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THE PRODUCTION OF SHOCK BY THE PROLONGED CONTINUOUS INJECTION OF ADRENALIN IN UNANESTHETIZED DOGS

N. E. FREEMAN, H. FREEDMAN AND C. C. MILLER

From the Harrison Department of Surgical Research, Schools of Medicine, University of Pennsylvania, Philadelphia

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Reduced plasma volume is recognized as the central feature of surgical shock. One of the hypotheses (5) advanced to explain the reduction is vasoconstriction which, if of sufficient intensity and duration, leads to anoxia of peripheral tissues, an increase in capillary permeability, and a loss of plasma from the blood stream into the tissues.

The vasoconstrictor concept of shock depends upon the demonstration of a decrease in circulating plasma volume resulting from prolonged vasoconstriction. The present experiments on trained normal dogs were undertaken to determine whether or not a reduction of plasma volume could be produced by prolonged intravenous injection of adrenalin in amounts sufficient to produce a marked reduction in the peripheral circulation.

METHODS. Adult dogs of 12 to 18.8 kgm. were used. They were trained to lie quietly on their sides and were found to remain in this position best while blindfolded. Blood pressure was recorded directly from the left femoral or left brachial artery by means of a cannula, inserted under local anesthesia, connected with a tambour manometer. The rate of blood flow in the right hind paw was measured with a plethysmograph according to the method of Freeman and Zeller (7). Hemoglobin concentrations were measured with a Sahli hemoglobinometer on blood obtained from the right ear. Hematocrit determinations were made on heparinized blood from the right jugular vein at the beginning of the experiment and again at the time when the second plasma volume determination was started.

The plasma volume determinations were made with the dye T-1824 (Gregersen, Gibson and Stead, 10; Gibson and Evans, 8) before the

adrenalin injection was started and again during the injection after one to one and one-half hours. In most of the experiments in which plasma volume was measured, the changes were also calculated from the deviation of the disappearance curve. Exactly 1 cc. of dye was injected into the right saphenous or left brachial vein. Ten to twenty-five minutes were allowed to elapse before taking the first sample. Specimens of blood were taken at regular intervals throughout the experiment in order to follow the changes in the dye concentration. Sufficient blood was taken without stasis from the right jugular vein to yield 2 cc. of serum. In some of the experiments this blood was immediately replaced by citrated blood from a normal dog. The concentration of dye in the serum was measured either spectrophotometrically or by means of a photometric colorimeter (Gibson and Evelyn, 9).

Just before the injection was started, the dogs were given atropin sulphate, 0.2 mgm. per kilogram intravenously, in order to prevent cardiac irregularity. Adrenalin hydrochloride (Parke, Davis and Company) diluted to 1:5000 in physiological saline was used in all but one of the

TABLE 1

DOG	SALINE		BLOOD PRESSURE		BLOOD FLOW		PLASMA VOLUME		
	Time	Total cc.	Initial	During saline	Initial	During saline	Initial	After saline	Per cent change
1	131	91.6	120	130	5.2	9.5		771	
2	78	69.0	115	135	10.0	9.0	879	854	−3
3	97	96.0	120	120	3.0	3.5	845	870	+3

experiments. Powdered adrenalin was made up in physiological saline solution to the same concentration in this exception (see table 2, dog 5). The adrenalin was injected at a constant rate of 0.0034 to 0.0164 mgm. per kilogram per minute. In three additional experiments, an equivalent amount of normal saline solution was injected to determine the effect of the intravenous injection of fluid alone. The injections were made by means of a ureteral catheter inserted under local anesthesia into a large superficial vein of the right foreleg or into the inferior vena cava by way of the left femoral vein. A constant rate of injection was maintained by the use of a Marey flask. The solution was displaced by liquid petrolatum.

Results. When normal saline was injected intravenously the dogs remained quiet, and there were no changes in the mucous membranes or extremities. None of them vomited, and there was no defecation. All recovered uneventfully, and were used in subsequent experiments.

There were no significant changes in blood pressure, blood flow, or in plasma volume, measured before and immediately after the injection. The results are shown in table 1.

Adrenalin. After all operative procedures, including the injection of atropin were completed and before the adrenalin injection was started, the dogs lay quietly with a normal respiratory rate and a rapid, but full, pulse. Shortly after the injection was begun, a moderate hyperpnea was noticed and the dogs became restless. Following this, vomiting frequently occurred.

Usually within ten minutes the mucous membranes of the mouth were seen to become pale and there was gradual progression to cyanosis. At the end of fifty to sixty minutes the clinical signs of shock were marked.

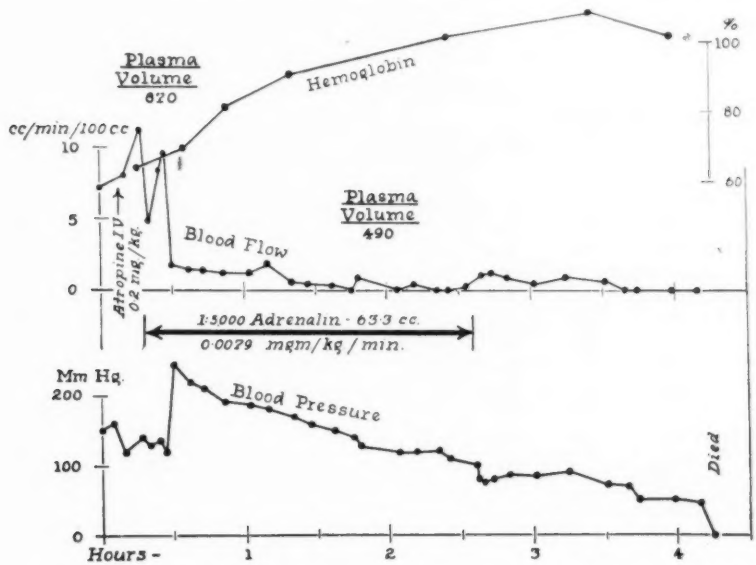

Fig. 1. Effect of prolonged injection of adrenalin (0.0079 mgm./kgm./min.) on blood pressure, blood flow, plasma volume and hemoglobin concentration of unanesthetized dog.

In addition to the pallor and cyanosis, the extremities and ears were cold and had a "doughy" feel. The pulse was thready, and the flow of blood in the ear veins was slow and the blood was dark. The animals were quiet and appeared exhausted but the eye reflexes were active throughout. Involuntary defecation occurred shortly before death.

The rectal temperature rose rapidly, but was prevented from going above 102°F. by wetting the dogs and cooling them with an electric fan.

When the adrenalin injection was started, the blood pressure rose to 250 to 370 mm. Hg. and the rate of blood flow through the paw decreased.

The flow was maintained at a low level during the adrenalin injection, and in most of the experiments it was below one cubic centimeter per minute per 100 cc. paw volume. The blood pressure declined slowly but was maintained throughout the period of injection at a level higher than the normal. The course of a typical experiment is shown in figure 1.

Determinations of plasma volume, made by the "direct" method, showed a fall of from 11 to 44 per cent, averaging 30.6 per cent. Hemoglobin determinations made at the same time indicated a 20 to 70 per cent concentration of the blood. There was no significant change in the plasma protein concentration during the adrenalin injection in the two experiments in which it was measured. The results of all the experiments with injection of adrenalin are summarized in table 2.

TABLE 2

DOG NO.	ADRENALIN	TIME OF INJECTION	BLOOD FLOW THROUGH PAW		PLASMA VOLUME			HEMOGLOBIN		
			Before adrenalin	During adrenalin	Before	After	Change	Before	After	Change
	mgm./ kgm./min.	*min.*					*per cent*	*per cent*	*per cent*	*per cent*
1	0.0116	130	10.5	0.5	876	553	−37	85	115	+38
2	0.0129	120	8.8	0.5	1102	758	−31	66	96	+45
3	0.0079	135	8.0	0.7	870	490	−44	64	109	+71
4	0.00465	190	9.6	2.3	1162	820	−29	60	102	+70
5	0.0164	100	9.9	1.9	1062	942	−11	51	61	+19
6	0.0111	126	15.0	3.0	1080	635	−41	98	118	+20
7	0.00766	180	17.2	0.9	627	384	−39			
8	0.0038	210	10.6	2.1	1390	1210	−13			
9	0.0034	166	5.0	1.5				100	166	+66
10	0.0085	132	11.5	0.5				64	87	+36
11	0.0125	66	11.5	0.5				83	136	+64
12	0.0102	141	27.0	0.75				122	168	+38
13	0.0045	143	25.2	1.0				104	152	+46

When the injection was stopped, the blood flow remained at a low level and the blood pressure fell. All but dogs 4 and 13 died or were sacrificed in a few minutes after stopping the adrenalin injection. These two dogs were given transfusions of citrated blood and 50 per cent glucose intravenously. In one hour after the transfusions the blood pressure had returned to the control level and the dogs recovered. The changes in one of these dogs are charted in figure 2.

In five experiments the changes in plasma volume observed with the direct method were followed concurrently by the "indirect" method of Gregersen. As shown in figure 3, according to the values calculated by the "indirect" method, the volume decreased from 1102 to 945 cc. A second

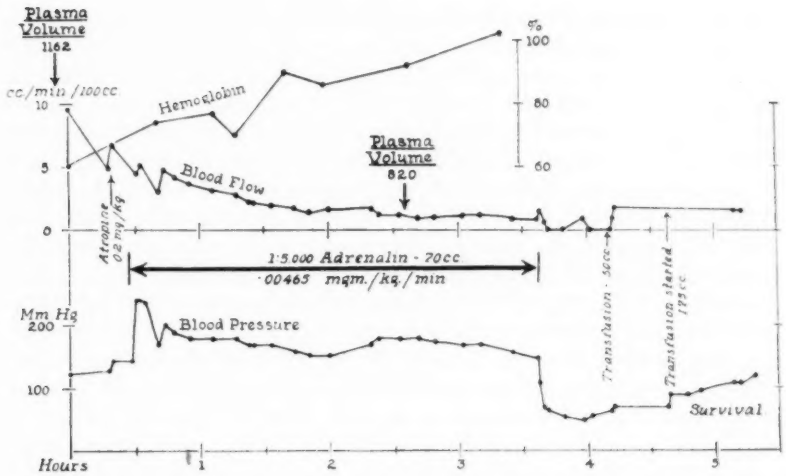

Fig. 2. Recovery of unanesthetized dog from shock produced by prolonged injection of adrenalin after transfusions of blood.

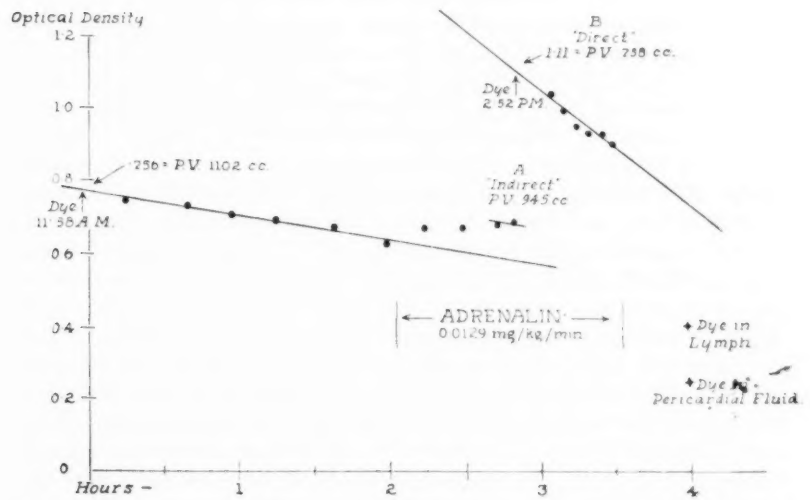

Fig. 3. Comparison of plasma volume changes after prolonged injection of adrenalin: a, by indirect method; b, by direct method after a second injection of dye. Adrenalin (0.0129 mgm./kgm./min.) injected intravenously between arrows. Crosses indicate concentration of dye in lymph from root of mesentery and fluid from pericardial sac.

"direct" determination with a second injection of dye, however, showed the plasma volume to be only 758 cc.

Autopsies were performed on all of the animals. Gross and microscopic changes were consistently observed. The thoracic cavities were empty of fluid, but there was a small amount of clear dye-stained fluid in the abdomen. The lungs were normal both grossly and on section, and showed no evidence of edema, but there were occasional areas of congestion.

The pericardial sac contained small to moderate amounts of dye-stained fluid but there was no evidence of tamponade. The heart was slightly enlarged, chiefly due to dilatation of the right ventricle. There were usually a few petechial subepicardial hemorrhages, and a moderate number of subendocardial hemorrhages over both right and left ventricles. The coronary sulcus was swollen and edematous, but there was no evidence of obstruction to the coronary flow. Section of the heart muscle showed only a small amount of edema.

The gastro-intestinal tract showed the most striking changes on gross examination. The intestines were soggy and the serous surfaces were pale. The lumen of the intestines contained blood-tinged fluid, and on one occasion contained dye-stained fluid. The duodenum consistently had numerous mucosal and submucosal hemorrhages and occasional hemorrhages were present in the stomach and ileum. Dye-stained fluid was obtained from the cisterna chyli on five occasions and in additional instances the mesenteric lymphatics also contained dye-stained fluid.

The liver and kidneys were congested and numerous areas of hemorrhage were present on section. The adrenal medullae were grossly hemorrhagic.

Discussion. The changes observed in the present series of experiments are in agreement with the results of other investigators. Bainbridge and Trevan (1), Erlanger and Gasser (3), Freeman (5), and Lamson and Keith (13) have reported a fall in plasma volume following the injection of adrenalin into anesthetized animals. Gregersen and Pinkston (11) and Hamlin and Gregersen (12), however, have pointed out that the anesthetic alone may cause a fall in plasma volume, and they found no change following injection of adrenalin into unanesthetized animals. They also criticized the method of plasma volume determinations used by Freeman and by Lamson and Keith on the grounds that insufficient time was allowed for mixing of the injected dye in the blood stream. The present series of experiments were carried out on dogs trained to lie quietly without anesthesia. The method of determining plasma volumes described by Gregersen, Gibson and Stead (10) and Gibson and Evans (8) with the dye T-1824 was used. With these methods the injection of adrenalin resulted in a marked fall in plasma volume. Certain observations in these experiments seem to indicate an explanation for the apparent difference in results.

Approximately the same quantities of adrenalin (0.0034 to 0.0164 mgm. per kgm. per min.) were used in these experiments as were injected by Gregersen and Pinkston (0.005 to 0.006 mgm. per kgm. per min.) and by Hamlin and Gregersen (0.005 to 0.035 mgm. per kgm. per min.) in experiments on normal unanesthetized dogs and cats. In our experiments, however, the injection was maintained for one to one and one-half hours before the dye for the second plasma volume determination was introduced. In the experiments reported by Gregersen and Pinkston and by Hamlin and Gregersen, the duration of the injection was less than thirty minutes in two-thirds of the experiments, and exceeded forty-five minutes in only one instance. It seems possible that the discrepancy between our results and those of Gregersen and Pinkston and Hamlin and Gregersen was due to the difference in the duration of injection.

In a personal communication Gregersen pointed out that his experiments on adrenalin injection into unanesthetized animals were designed to demonstrate alterations in normal physiology in contrast to the pathological changes produced in our experiments. None of his animals showed the clinical changes of extreme pallor and cold extremities indicative of intense vasoconstriction, and apparently this effect had not been attained or maintained.

In the present experiments a discrepancy was found between values for the plasma volume determined by the "direct" and "indirect" methods. This discrepancy may possibly be explained on the basis of changes occurring in the circulatory system during the adrenalin injection. The validity of the "indirect" method depends on the assumption that the rate of disappearance of the dye from the blood stream remains constant before and during the injection of adrenalin. However, capillary permeability is probably increased during the vasoconstriction produced by the adrenalin. Landis (14) has shown by direct observation of the capillaries that obstruction to the blood flow and the consequent anoxia of the endothelium results in increased permeability and a loss of both fluid and proteins into the surrounding tissue. For example, he demonstrated (15) that the arterial spasm of Raynaud's disease is followed by an increase in fluid in the tissue spaces. It was observed by Evans and Gibson (4) that where the permeability of the endothelium is altered, the resulting exudates contain high concentrations of the previously-injected dye. Post mortem observations in our experiments have shown dye-stained fluid in the tissue spaces, in the pericardial sac, in the cisterna chyli, and in the lumen of the intestines. Further evidence of rapid loss of dye from the blood stream is seen in the steeper slope of the disappearance curve of the dye following the second injection of dye (fig. 3).

The changes in the heart consistently observed were dye-stained fluid in the pericardium and in the tissue of the coronary sulcus, and subendocardial

hemorrhages. We are unable to explain the mechanism of these changes. However, that they were not significant in causing the death of the dogs was shown by the fact that two dogs recovered following treatment. Post mortem studies in one of these dogs a week later showed evidence of old hemorrhages. Although an increase in venous pressure did occur during the adrenalin injection, as shown by the distended neck veins, it was probably not an important factor in the decrease in plasma volume since the plasma protein concentration was not altered.

A relatively large amount of adrenalin was used, much larger than any amount which could be secreted physiologically. The dosage was chosen to reduce the peripheral blood flow to below 1.5 cc. per minute per 100 cc. paw volume. It was found by Freeman, Shaffer, Schecter and Holling (6) that if the flow was maintained below this level by repeated hemorrhages, shock was produced. The present experiments appear to indicate that if the blood flow is severely reduced from prolonged vasoconstriction due to the injection of adrenalin, shock is produced. The alterations in the physiology leading to the decrease in plasma volume are probably initiated by the anoxia of the peripheral capillaries. The effect of this anoxia is to increase the capillary permeability leading to a loss of fluid and proteins into the surrounding tissues. The blood pressure is maintained by the vasoconstriction but declines slowly due to the loss of circulating fluids. It is the combination of the loss of circulating fluids and the peripheral vasoconstriction which produces the clinical appearance of shock. The central blood flow is maintained as long as the blood pressure remains at a fair level, and the animals remain alert with active reflexes throughout. When the adrenalin is stopped and the blood vessels are permitted to relax, reflex vasoconstriction, in response to the lowered pressure, is insufficient to maintain adequate circulation to the vital centers because of the fall in circulating blood volume.

That the state of the animals during the adrenalin injection was due primarily to the loss of circulating fluids was shown in the two instances in which blood transfusion and intravenous hypertonic glucose solution restored the dogs to normal. Within an hour after this treatment was instituted, the blood pressures had risen to normal levels and the dogs recovered. In another instance, the hemoglobin concentration, which had increased 46 per cent, returned within an hour of treatment to its normal level.

Post mortem studies showed an abnormal amount of fluid outside of the vascular system—in the lymph, in the pericardial sac, in the lumen of the intestine, in the peritoneal cavity, and in the tissue spaces. This finding is considered to be significant. Blalock (2) has suggested that the fluid loss leading to shock is to be attributed to the local congestion, edema, and hemorrhage in the traumatized portion of the body. Frequently, however,

in clinical cases of shock, no such local trauma is discernible, but there is still evidence of a loss of fluid from the circulating blood. The location of this lost fluid has not yet been demonstrated. The findings presented suggest a possible explanation: that the fluid is widespread in the tissue spaces and that some of it is lost through the intestinal mucosa into the lumen of the digestive tract. Improvement following adequate treatment is probably aided by the absorption of this fluid back into the circulating blood.

The changes observed in the experimental animals are much the same as those presented by the human patient in a state of surgical shock. It should be emphasized, however, that there is no evidence that clinical shock in humans is a result of the overproduction of adrenalin. In these experiments, adrenalin was used only as a method of producing prolonged vasoconstriction in the absence of trauma and hemorrhage.

SUMMARY

1. In thirteen experiments on unanesthetized dogs, vasoconstriction produced by the continuous intravenous injection of adrenalin solution (0.0034 to 0.0164 mgm. per kgm. per min.) for a period of one to one and one-half hours resulted in a decrease in plasma volume and a state of shock.

2. Continuous injection of an equivalent amount of normal saline for similar periods caused no change in the plasma volume.

3. The calculation of changes in plasma volume from the deviation of the disappearance slope of the dye T-1824 during the production of shock is not satisfactory because the dye is not retained in the circulating blood.

4. Post-mortem studies suggest that the fluid lost from the circulating blood in shock is distributed through the tissue spaces, and that some of it passes through the intestinal mucosa into the lumen of the digestive tract.

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THE MECHANISM OF THE PRODUCTION OF BRAIN DAMAGE DURING INSULIN SHOCK[1]

DAVID B. TYLER

From the University of Southern California, Los Angeles

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If inhibition of cerebral respiration[2] is the principal, if not the sole cause of the changes which occur during insulin shock, certain inconsistencies must be accounted for. On the basis of experimental work, numerous investigators have suggested other factors to be the cause of brain damage during acute hypoglycemia. These may be summarized as follows: 1. Insulin, per se, is toxic to the brain cell (1, 2). 2. Convulsions cause some changes that produce brain damage (3). 3. Hypoglycemia depresses the metabolism of the brain. This depression causes a decreased oxygen uptake of the brain (4, 5). 4. Insulin shock impairs circulation.

It was shown previously that it is necessary to maintain cats for a definite time in the "medullary phase" of insulin shock (phase IV) to produce brain damage (6). However, an analysis of those results reveals a relationship between the amount of insulin given and the incidence of brain damage.

This report deals with the effects of temperature, sodium pentobarbital, and varying insulin doses on the production of brain damage. Considering the widespread use of insulin shock therapy, and the numerous accidents which occur during its use, a study of this nature seems desirable.

METHODS. Only adult cats weighing 3 kgm. or more, and not previously treated with insulin, were used in these experiments. The method used to produce brain damage has been described in earlier reports (6, 7, 8).

To determine the duration of action of various doses of insulin, blood sugars (Folin-Wu) were taken at intervals. The time for the blood sugars

[1] The author is indebted to Dr. C. H. Thienes for use of the laboratory facilities of the Department of Pharmacology, School of Medicine, University of Southern California, and to the National Youth Administration for the services of Messrs. R. Bernhard, R. Clingman, C. Berry and G. Hogan.

Insulin was kindly furnished by Eli Lilly & Company. Sodium pentobarbital was supplied as Veterinary Nembutal by Abbott & Company.

[2] Inhibition of cerebral respiration refers to the impaired ability of the brain to remove oxygen from the arterial blood during hypoglycemia even though the O_2 saturation may be at normal or near normal levels.

to return to normal was considered the duration of action of the given dose.

In one group of animals 20 u. of insulin per kilogram of body weight were given subcutaneously. When the first myoclonic jerk occurred, 10 mgm. per kgm. of body weight of sodium pentobarbital were given intraperitoneally. The administration of this drug abolished the characteristic progression of symptoms that occur during insulin shock. The only remaining symptoms that could serve as an index of the neurological state of the animal were the respiration and pulse rate. When these became very slow and irregular, the animals were considered to be in the medullary phase. If symptoms of complete circulatory and respiratory collapse became evident, small amounts of glucose were given intraperitoneally, as described in previous reports. In these animals, the hypoglycemias were terminated after they had spent a total of 150 minutes in the medullary phase.

Animals which exhibited the irreversible clinical symptoms of "decortication" or "decerebration," as described by Ziskind and Tyler (7), were considered to have brain damage.

RESULTS. 1. *Sequence of symptoms in the cat during insulin shock.* In cats not receiving sodium pentobarbital the symptoms are similar to those described for humans by Angyal (9) and Frostig (10), the essential difference being the time of occurrence of the various phases. In cats not receiving insulin previously the time before the onset of convulsions increases with the size of the insulin dose given (6). This paradoxical action of insulin does not hold when insulin has been given daily. If the medullary phase occurs, it generally starts from the 6th to the 8th hour after insulin has been given. The number of cats which show medullary symptoms increases progressively with increasing doses (column 2 of table 1). These symptoms rarely occur with doses of 5 u./kgm. or less.

2. *Duration and intensity of action of different doses of insulin.* With 10 u./kgm. (average dose 33 u.), the duration of action ranges from 10 to 14 hours, while with 20 u./kgm. (average dose 70 u.), it is greater than 18 hours (column V of table). These results are in agreement with those found for depancreatized dogs (11). However, the blood sugar level at any instant during hypoglycemia is not an index of the intensity of action at that moment, for the obvious reason that its fall is definitely limited to 0 mgm. per cent. Drury and Greeley (11) measured the intensity of action by means of the ability of various insulin doses to dispose of injected glucose while the blood sugar was maintained at physiological levels. They found that within one hour after giving 10 u. of insulin, a peak glucose disposing ability of 4 to 6 grams per hour was attained by the animal. This gradually tapered off so that at the 3rd hour only 2 grams per hour could be disposed of, and by the 8th hour no glucose

disposing ability was left. With 100 units of insulin a peak of 11 to 13 grams per hour was reached in about 60 minutes. By the 8th hour it fell to 4 to 7 grams per hour, by the 12th hour it could still dispose of from 2 to 4 grams per hour, and, in some instances, the glucose disposing ability still persisted after the 14th hour.

These results indicate that even though the blood sugar level may be the same for different doses of insulin, the intensity of action at the time is dependent upon the amount of insulin initially given. The significance of this in the production of brain damage is discussed below.

TABLE 1

DOSE	HOURS AFTER ADMINISTRATION OF INSULIN THAT MEDULLARY SYMPTOMS OCCUR	NUMBER OF CATS MEDULLARY PHASE OCCURS	INCIDENCE OF BRAIN DAMAGE	DURATION OF ACTION OF INSULIN	BLOOD SUGAR RANGE AT 8TH HOUR	ESTIMATED INTENSITY OF INSULIN ACTION AT 8TH HOUR†
Units per kilogram			*per cent*	*hours*		*grams*
1.0 Av. dose = 3.4		0/8	0	3–5½		0.0
5.0 Av. dose = 16	6¾	1/7	0	6–10	20–43‡	0.+
10.0 Av. dose = 35	7–8	4/7	30*	10–14	18–28	3.0
15.0 Av. dose = 52	6¾–7½	6/6	33*	12–16+	20–30	4.5
20.0 Av. dose = 71	7–8	20/20	62*	18+	18–29	7.0

* From previous report of Tyler and Ziskind.

† Calculated from data of Drury and Greeley, and expressed as the amount of injected glucose that the remaining insulin can dispose of per hour.

‡ Taken on cats still in coma at 8th hour.

3. *The effects of sodium pentobarbital on brain damage.* Of 18 cats given 10 mgm./kgm. of sodium pentobarbital when the first myoclonic jerk or convulsion occurred, 6 died before the hypoglycemias could be terminated. The 12 remaining cats were terminated after they had been in the medullary phase for a total of 150 minutes. This length of time invariably produced brain damage in cats not given sodium pentobarbital, and the resulting preparations usually lived about 48 hours after termination of the hypoglycemia (7). All these 12 cats remained in a protracted coma of from 2½ to 6 days after the hypoglycemias were terminated. Four of these cats died on the 3rd day and 3 on the 4th day without regaining con-

sciousness. One regained consciousness on the 3rd day, showed symptoms of sub-cortical damage, and died on the 5th day. Two cats regained consciousness on the 2nd and 3rd days, and showed symptoms of cortical damage. These cats were sacrificed 21 days later. The 2 remaining cats recovered consciousness on the 3rd and 6th days after termination, and although very sick from a pulmonary infection, did not show any clinical signs of brain damage.

4. *The effects of temperature on the production of brain damage.* When massive doses of insulin are given to a cat, its body temperature usually falls about 6°C. by the time coma sets in. In these "hypothermic" animals brain damage occurs only in the medullary phase (6). If the fall in temperature is prevented, brain damage is produced much more readily. When the body temperature is maintained from 38° to 40°C. the animal gives the appearance of suffering from a severe anoxia. The hypoglycemic symptoms proceed very rapidly, and the animal sinks into the medullary phase suddenly. The character of this phase is altered also. Instead of the usual slow pulse, it may be extremely high. The respiration is essentially Cheyne-Stokes in character. The corneal and pupillary reflexes, as in the hypothermic cat, are absent, and the animal is completely flaccid. Death occurs much sooner in these cats than in the hypothermic cat. If the temperature is raised to above 40°C. during hypoglycemia, death may occur shortly after the animal has had a convulsion. In the hypothermic cat death rarely occurs until the animal has been in the medullary phase for a considerable period. In 3 cats, the body temperature was maintained between 40° and 42°C. by means of a hot pad. The hypoglycemias were terminated one hour after coma set in. All 3 cats showed unmistakable symptoms of cortical damage.

Discussion. From the work of Himwich et al. (5) it is clear that the progression of symptoms during insulin shock is associated with a decreasing ability of the brain to take up oxygen. In other words, as the inhibition of brain respiration increases in severity, lower and lower levels of the brain are affected, depending upon the oxygen need of the various regions. It has been shown that the medulla normally has the lowest metabolic rate (12) and requires the least amount of oxygen per gram weight of tissue (13). It may be assumed, then, that the inhibition of oxygen uptake by the brain must be greatest when the medullary symptoms occur. These symptoms generally appear about the 7th hour (column 1). From the table it can be observed that neither the differences in the duration of action of the various doses of insulin nor the blood sugar levels at the 7th or 8th hours can account for the differences in the incidence of brain damage or the number of animals which show medullar symptoms during insulin shock. In this respect it is found that with 5 u./kgm. the duration of hypoglycemia is long enough to produce medullary symptoms, or even

brain damage, *provided* hypoglycemia, per se, is the only factor involved. Yet, with this dose, only one cat showed medullary symptoms and no irreversible brain damage occurred. On the other hand, with 20 u./kgm. all cats showed medullary symptoms and brain damage was produced in 63 per cent of them. It was pointed out above that the blood sugar level, per se, following massive doses of insulin, is meaningless since it does not give an indication of the intensity of action of insulin at that instant. This "time-activity-factor" is dependent upon the amount of insulin initially given (11). Calculating from the results of Drury and Greeley, we have listed in column VI the estimated amount of insulin action existing 8 hours after injection of different doses. The 8th hour was chosen for these estimations since in the cat it is necessary that the medullary phase be maintained for over an hour to produce brain damage (6). The estimated insulin activity remaining at the 8th hour reveals that with 5 u./kgm., less than 1 gram of glucose disposing ability remains; with 10 u./kgm., it is about 3.0 grams/hour; with 15 u./kgm. it is 4.5 grams/hour, and with 20 u./kgm., it is 7.0 grams/hour. These differences in intensity of action undoubtedly account for the differences in the frequency of medullary symptoms, and also the incidence of brain damage.

Why the inhibition of cerebral respiration is not severest within one hour after insulin is given (for at that time the greatest intensity of action occurs) may probably be attributed to the glycogen reserve in the brain. Although this storage is meager, it is apparently sufficient to take care of the brain's requirement for a few hours (14).

Inasmuch as medullary symptoms indicate an intense depression of the oxygen uptake by the brain (5), and since the frequency of occurrence of these symptoms increases with larger doses of insulin, it may be concluded that the depression of oxygen uptake by the brain ultimately produced depends upon the intensity of insulin action and, thus, on the amount of insulin initially given.

These correlated facts, experimentally established, can account more satisfactorily for the greater incidence of brain damage which occurs with larger doses of insulin than an unknown quality based on the assumption that insulin, per se, is toxic to the brain cell.

Barbiturates are known to depress cerebral metabolism (15). Although it appears from the above results that the degree and incidence of brain damage are less in nembutalized cats, this point is not conclusive. However, despite the occurrence of the prolonged comas, the survival time of the preparations is increased when sodium pentobarbital is given. This drug prevents insulin convulsions but, nevertheless, brain damage will occur. This confirms the findings of others who showed that convulsions were not essential in the production of brain damage (16, 1).

The clinical symptoms of circulatory collapse first occur during the medullary phase. This is as would be expected since the cardiac and respiratory centers in the medulla should be affected by the decreased oxygen uptake at that time. The circulatory collapse, it appears then, is a result of the inhibition of cerebral respiration on those centers, and is an indication that brain damage is taking place.

The mechanism generally accepted to be responsible for the decreased oxygen uptake by the brain during hypoglycemia is based on the observations that the brain can oxidize only glucose or carbohydrate, and when the supply of this foodstuff is curtailed or stopped, the oxygen uptake of the brain is consequently diminished (20, 17, 4, 5). Himwich and his collaborators have further established the close correlation between glucose absorption and the oxygen uptake by the brain. From their work it appears evident that glucose is vitally concerned with cerebral respiration.

If the inhibition of cerebral respiration that occurs during hypoglycemia produces a state very similar to that caused by anoxia, it should be expected that conditions which would lower the oxygen need of the brain should increase the resistance of the brain to damage during insulin shock. In this respect it is found that when the body temperature is allowed to fall during hypoglycemia, the medullary phase has to be maintained for a longer time, in order to produce brain damage, than in those animals whose body temperature is kept at normal levels. Furthermore, it has been noted that precooled animals survive longer when subjected to low partial pressures of oxygen (18) or after receiving lethal doses of insulin (19). Lowering the body temperature lowers the metabolic rate and hence the oxygen need of the tissue. The apparent protective action of nembutal may possibly be explained by the depressed cerebral metabolism that results when this drug is given. Conversely, increased body temperature increases the oxygen need of the brain, and the time required to produce brain damage during insulin shock is shortened.

SUMMARY

Some factors influencing the production of brain damage during insulin shock have been studied. It has been found that:

1. It is necessary to maintain cats in the medullary phase of insulin shock to produce clinical symptoms of brain damage.

2. The medullary phase occurs more often and the incidence of brain damage is greater with increasing doses of insulin. This is attributed to the "time-activity" factor of insulin action whereby the intensity of action of a single dose of insulin at any instant after injection is dependent on the amount of insulin initially given.

3. The length of time that cats must be maintained in the medullary phase of insulin shock to produce brain damage is a function of the oxygen need of the brain.

4. When the body temperature is maintained at normal or above normal levels the medullary phase occurs sooner, the time the animal must be kept in this phase to produce brain damage is shortened and the incidence of brain damage is greater. Low body temperature produces the opposite results.

5. Although prolonged comas result when sodium pentobarbital is given during insulin shock the survival time of the animals is increased.

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THE METABOLISM OF CATS UNDER CHLORALOSE ANESTHESIA, WITH SPECIAL REFERENCE TO OXYGEN CONSUMPTION

FRED R. GRIFFITH, JR., F. E. EMERY AND JULIA E. LOCKWOOD

From the Department of Physiology, University of Buffalo, Buffalo, N. Y.

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This is to report the variability of, and degree of correlation between oxygen consumption, carbon dioxide output, respiratory quotient, pulmonary ventilation, systolic blood pressure, heart rate, blood sugar and lactic acid concentrations of chloralose anesthetized cats. Such a compilation, though pedestrian and uninspired, may be useful either for: establishment of statistically valid norms and standards as basis for evaluation of experimental results under this commonly used anesthetic; or, perhaps more important, for the insight which is provided, especially by their interrelationships, as to the normality of physiological state and adjustment under anesthesia. In both respects principal interest attaches to oxygen consumption as an index of the total metabolic condition; and it will be analyzed in further detail as to the effect upon it of 1, sex; 2, season; 3, body temperature; 4, body size (weight); and 5, the relation of it and respiratory quotient to values for normal, unanesthetized animals.

PROCEDURE. The data are derived from 50 male and 34 non-pregnant female cats. At the time they were used they had been in the school pens for variable periods of time, but usually not more than a few days. In preparation for experiment they were brought to the laboratory 16 to 18 hours after the last meal and anesthetized with chloralose, 0.1 gram per kilogram, injected under the skin of back or flank. As soon as quiet they were placed on an electrically heated holder and carefully attended to prevent undue lowering or fluctuation of body (rectal) temperature.

Further preparation involved very little trauma, viz., careful insertion of: a tracheal cannula; a blood-sampling cannula in one carotid artery; a blood-pressure cannula in the other carotid; and a cannula in a superficial branch of one femoral vein. Following these simple operative procedures a rest period of at least 15 to 20 minutes was allowed for stabilization before observations were begun.

With 57 of the 84 animals two determinations of respiratory metabolism were made 15 to 20 minutes apart; the second of these duplicate determinations plus the single determination on the remaining 27 animals constitute the 84 observations providing data for part II of table 1. Com-

TABLE 1

Data of cats under chloralose anesthesia

	I				II					
	INTRA-INDIVIDUAL VARIABILITY, COMPARISON OF DUPLICATE DETERMINATIONS, 15–20 MINUTES APART				INTER-INDIVIDUAL VARIABILITY					
	Number	Averages		Diff. per cent of mean	Number	Range	Mean	Standard deviation	Coefficient of variation*	Coefficient of correlation ×
		1st	2nd							
1. Body weight (kgm.)					84	1.8–4.7	3.0	0.62	20.5	-0.102 ± 0.072 × Pulmonary ventilation -0.099 × O_2 consumption per kilo per min. (partial correlation with temp. constant) -0.029 ± 0.080 × O_2 consumption (entire data) $+0.071 \pm 0.074$ × Blood sugar $+0.114 \pm 0.075$ × Blood lactic acid $+0.123 \pm 0.072$ × Blood pressure $+0.169 \pm 0.070$ × Body temperature
2. Time after injection of chloralose (min.)					84 (73)	60–245 (110–190)	150	32.5	21.7	-0.264 ± 0.068 × Blood pressure -0.087 ± 0.074 × Oxygen consumption -0.085 ± 0.074 × Blood lactic acid $+0.018 \pm 0.074$ × Heart rate $+0.250 \pm 0.072$ × Blood sugar
3. Rectal temperature (°C.)	57	37.7	37.7	0	84 (63)	36.6–38.8 (37.5–38.5)	37.8	0.49	1.29	-0.127 ± 0.072 × Blood pressure $+0.027 \pm 0.076$ × Blood sugar $+0.169 \pm 0.070$ × Body weight $+0.203 \pm 0.071$ × Heart rate $+0.313 \pm 0.070$ × Blood lactic acid $+0.358 \pm 0.064$ × O_2 consumption/kgm. body wt. $+0.382 \pm 0.062$ × Pulmonary ventilation $+0.382 \pm 0.062$ × Respiratory quotient $+0.424 \pm 0.060$ × CO_2 output/kgm. body wt.

4. Blood pressure (mm. Hg)					84	85–235	150	30.8	20.4	−0.264 ± 0.068 × Time after chloralose −0.264 ± 0.069 × Heart rate −0.133 ± 0.071 × Pulmonary ventilation −0.127 ± 0.072 × Rectal temperature −0.016 ± 0.076 × Blood lactic acid +0.086 ± 0.074 × Oxygen consumption +0.123 ± 0.072 × Body weight
5. Heart rate (beats/minute)					84	95–285	190	34.4	18.0	−0.264 ± 0.069 × Blood pressure +0.018 ± 0.074 × Time after chloralose +0.153 ± 0.070 × Oxygen consumption +0.203 ± 0.071 × Rectal temperature
6. Blood sugar (mgm. per cent)	36	125	129	1.6	78	75–265	145	37.2	25.5	+0.027 ± 0.076 × Rectal temperature +0.100 ± 0.077 × Blood lactic acid +0.112 ± 0.076 × Respiratory quotient +0.250 ± 0.070 × Time after chloralose +0.306 ± 0.070 × Oxygen consumption
7. Blood lactic acid (mgm. per cent)	36	10.8	9.3	7.5	78	4–22	11.2	3.97	36.1	−0.016 ± 0.076 × Blood pressure +0.086 ± 0.076 × Respiratory quotient +0.100 ± 0.077 × Blood sugar +0.232 ± 0.073 × Pulmonary ventilation +0.401 ± 0.067 × Oxygen consumption
8. Pulmonary ventilation (cc./kgm./min.) (0°, 760 mm.)	57	103	102	0.5	84	54–192	107	30.6	28.6	−0.102 ± 0.072 × Body weight −0.133 ± 0.071 × Blood pressure +0.232 ± 0.073 × Blood lactic acid +0.382 ± 0.062 × Rectal temperature +0.457 ± 0.057 × Respiratory quotient +0.729 ± 0.038 × Oxygen consumption +0.781 ± 0.027 × Carbon dioxide output
9. Respiratory quotient	57	0.735	0.741	0.4	84 (72)	0.64–0.86 (0.70–0.86)	0.745	0.048	6.49	+0.086 ± 0.076 × Blood lactic acid +0.112 ± 0.076 × Blood sugar +0.230 ± 0.070 × Oxygen consumption +0.457 ± 0.057 × Pulmonary ventilation +0.532 ± 0.055 × Carbon dioxide output

* Coefficient of Variation = $\frac{\text{Standard Deviation}}{\text{Mean}} \times 100$.

TABLE 1—*Concluded*

	I				II					
	INTRA-INDIVIDUAL VARIABILITY. COMPARISON OF DUPLICATE DETERMINATIONS, 15–20 MINUTES APART				INTER-INDIVIDUAL VARIABILITY					
	Number	Averages		Diff. per cent of mean	Number	Range	Mean	Standard deviation	Coefficient of variation*	Coefficient of correlation ×
		1st	2nd							
10. Carbon dioxide output (cc./kgm./min.)	57	4.65	4.63	0.2	84	2.7 7.7	4.81	1.05	21.9	+0.424 ± 0.060 × Rectal temperature +0.532 ± 0.055 × Respiratory quotient +0.781 ± 0.027 × Pulmonary ventilation +0.948 ± 0.014 × Oxygen consumption
11. Oxygen consumption (cc./kgm./min.)										
(1) Males					(50)	(4.6 9.3)	(6.59)	(1.25)	(19.0)	+0.948 ± 0.014 × Carbon dioxide output
(2) Females					(34)	(3.7 8.4)	(6.14)	(1.17)	(19.1)	+0.729 ± 0.038 × Pulmonary ventilation
(3) Total	57	6.35	6.24	0.9	84	3.7 9.3	6.43	1.23	19.2	+0.401 ± 0.067 × Blood lactic acid +0.358 ± 0.064 × Rectal temperature +0.306 ± 0.070 × Blood sugar +0.230 ± 0.070 × Respiratory quotient +0.153 ± 0.072 × Heart rate +0.086 ± 0.074 × Blood pressure −0.029 ± 0.080 × Body weight, entire series 84 observations −0.083 ± 0.084 × Body weight, within temperature range 37.5 38.5 C. −0.097 × Partial correlation with weight for the entire group with temperature constant −0.087 ± 0.074 × Time after injection of chloralose
12. Composition of expired air (per cent)										
(1) Oxygen	57	14.86	14.88		84	12.05 17.76	14.98	1.10	7.3	
(2) Carbon dioxide	57	4.68	4.72		84	2.68 6.80	4.62	0.76	16.4	

parison of the first and second determinations, when two were made, provide the data on intra-individual variability of part I of the table. Pulmonary ventilation and respiratory metabolism were determined by collection and analysis of expired air.

At the conclusion of the second metabolism determination when two were made, or of the single measurement, when there was only one, record was obtained of carotid blood pressure and heart rate by mercury manometer connected with the carotid pressure cannula. These 84 determinations are thus synchronous with the 84 measurements of respiratory metabolism of part II of the table and are recorded with them there.

With 78 of the 84 animals a blood sample of 2 cc. was taken just preceding the blood pressure determination; estimation of blood sugar and lactic acid concentrations derived therefrom are thus synchronous with the other data of part II of the table and are there recorded. An additional blood sample was also taken at the conclusion of the first measurement of respiratory metabolism with 36 of the 57 animals on which this duplicate determination was made. These 36 pairs of duplicate samples, taken 15 to 20 minutes apart, provide the data on intra-individual variability of blood sugar and lactic acid of part I of the table. Analyses were made on Folin-Wu tungstic acid filtrates; sugar by the method of Hagedorn and Jensen; lactic acid by that of Friedemann, Cotonio and Shaffer.

Results. *Intra-individual stability.* The question that comes first to mind in consideration of metabolism under anesthesia is whether, at the time of the observations, absorption, excretion or destruction of the anesthetic are sufficiently balanced or uniform to permit establishment of a physiological steady state. Most of these determinations were made within the period, 110 to 190 minutes, i.e., essentially during the third hour after subcutaneous injection of the chloralose; and the data of part I of table 1 show that at this time there was practically no change during a 15 to 20 minute interval in any of the variables measured. The data of part II of the table may, therefore, be accepted as characteristic of a stabilized narcotized condition.

Inter-individual variability. All measures of dispersion (range; standard deviation; coefficient of variation) of part II, table 1, are large. Body weight, the time after injection of anesthetic when observation was made, and body temperature, with proper selection and care could have been controlled within narrower limits. It is interesting to see to what extent variation of these may be responsible for the high degree of variability of the others.

Body weight as an index of size might be expected to influence oxygen consumption per unit weight; or, as index of age, blood pressure and perhaps, composition. In so far as degree of correlation (table 1, part II) permits causal inference, it may be eliminated as being responsible in

any significant degree for the wide variability shown by these data. This is not to say that in normal animals or under other conditions it might not be decisive, at least for certain functions; it is merely to point out that under the conditions of these experiments other factors must have played a far more disturbing rôle. The small negative correlation with oxygen consumption per unit weight, which only in sign is indicative of a relationship which might have been expected to be much more pronounced will be referred to later in more detail.

Time after injection of the chloralose when the observations were made might also be suspected as an underlying cause of variability through variation in depth of narcosis. Again, as judged by the coefficients of correlation (table 1, part II) it can have played but a small part. As might have been expected, blood pressure shows a barely measurable tendency to fall, and blood sugar to rise as narcosis deepens or is prolonged. But all correlations are too small to warrant belief that significantly greater uniformity would have been obtained had all observations been made at the end of exactly the same duration of anesthesia.

Body temperature, on the other hand, for uniformity of result, should have been controlled within narrower limits; blood lactic acid level, respiratory metabolism and related volume of pulmonary ventilation are all, according to statistical measure (table 1, part II), affected by it. Though significant, however, all correlations are too small to permit belief that any large part of the observed variability between different animals is due to this cause alone. The fact that these observations were made over the body temperature range observed here permits calculation of temperature coefficients for the functions that are significantly affected. This will be developed for the rate of oxygen consumption below.

From the preceding it may be concluded that variability of these extraneously controllable factors contributed but little to the observed large intrinsic variation of physiological functions in these animals. Next in interest would be to discover, if possible, whether some one disturbing factor in turn affected others to abnormal variability.

Physiological interrelationships under anesthesia are normal, would seem the legitimate conclusion from the observed correlations of which the nearly one-to-one relationship between oxygen consumption and carbon dioxide is pivotal, as the latter might so easily be falsified. The high degree of correlation of carbon dioxide output also with pulmonary ventilation and respiratory quotient would be expected in any case; but is apparently conditioned normally, here, since neither of the last two is significantly related to blood lactic acid level. Nor is the reciprocal relation of pulmonary ventilation to blood pressure large enough to warrant suspicion of suppression of the former from this cause sufficient to interfere with adequate oxygen intake and carbon dioxide output.

With this basis for believing oxygen consumption, however variable, a valid measure of the true metabolic rate of these anesthetized animals, justification is provided for further analysis with respect to the effect upon it of 1, sex; 2, season; 3, body temperature; 4, body size (weight); and finally, 5, its relation, together with that of respiratory quotient, to values for normal, unanesthetized animals.

1. Sex. The influence of this factor persists under anesthesia and therefore contributes something to the range of variability of the group as a whole. For the 50 males the range and average are, respectively, 4.6 to 9.3, and 6.59 cc. per kilo per minute; for the 34 females the corresponding figures are 3.7 to 8.4, and 6.14. The variability as measured by the coefficient of variation is practically the same for each sex, being 19.0 for the males and 19.1 for the females.

2. Season. Some of the variability of the group as a whole is apparently due to the fact that observations were made throughout the year except July and August. Thus the average rates of oxygen consumption, cc. per kilo per minute, with the number of determinations (in parentheses) are: winter, 5.53 (19); spring, 6.95 (38); June, 7.07 (12); autumn, 5.62 (15). The average rectal temperatures for the same seasonal groups are: 37.90; 37.91; 38.05 and 37.36°C.; therefore with the exception of the determinations made in autumn the seasonal difference is independent of this factor; and even for this group the temperature difference is not sufficient, as will appear from what follows, to explain all the decrease in metabolic rate.

This seasonal variation is shown by both sexes; thus, again, rates of oxygen utilization in cubic centimeters per kilo per minute, with the number of determinations (in parentheses) are:

Males: winter, 5.66 (9); spring, 6.93 (27); June, 7.4 (8); autumn, 5.40 (6).

Females: winter, 5.41 (10); spring, 7.00 (27); June, 6.40 (4); autumn, 5.77 (9).

The data are apparently insufficient to decide precisely whether maximum is in spring or early summer, or minimum in autumn or winter, but there would seem to be no doubt of a major cycle throughout the year, and which in these laboratory-confined animals agrees more nearly with that observed in human subjects in bed in the morning without previous immediate exposure to the weather (Gustafson and Benedict, 1928; Lockwood and Griffith, 1938) than with that of those suffering such exposure on the way to the laboratory before measurement (Griffith et al., 1929a). Also, for similar variation in the blood pressure of unanesthetized dogs see Hamilton et al., 1940.

3. Body temperature. The rectal temperatures of these animals varied from 36.6 to 38.8°C.; with 63, or 75 per cent of them within the range of a

single degree, 37.5 to 38.5°C. The average temperature for the entire group is 37.8° which is 0.9°C. below the normal for the cat, 38.7°C.

The coefficient of correlation between rectal temperature and rate of oyxgen consumption is $+0.358 \pm 0.064$; and the regression equation derived from this is:

$$\text{cc. } O_2 \text{ per kilo per minute} = (0.904 \times \text{T°C.}) - 27.8$$

According to this, over the two-degree range from 36.6 to 38.6°C., which practically corresponds with the extremes observed in this work, average oxygen intake should vary from 5.3 to 7.1 cc. per kilo per minute; and anesthetized animals held at the normal temperature (for the cat) of 38.7°C., should have an average oxygen consumption of 7.18 cc. per kilo per minute. This will be reverted to below in comparison of these with normal animals.

4. Body weight. Until the test was made it was expected that a good deal of the variability of oxygen consumption per unit of body weight was probably due to use of this convenient but supposedly unphysiological standard of reference. Analysis shows, however, that this has little or nothing to do with the wide variability of result and that there is practically no tendency within this group of anesthetized animals for metabolic rate per unit of weight to be consistently greater in small than in large animals. Thus within this weight range of 1.8 to 4.7 kilos, the coefficient of correlation between weight and oxygen utilization per unit of weight is only -0.029 ± 0.080. Aside from the anticipated negative sign this is numerically too small to have significance.

The relationship is slightly but not significantly improved if allowance is made for body temperature. Thus if all observations at extreme temperatures are eliminated and the correlation is determined only for those (63 in number) within the single-degree range of 37.5 to 38.5°C., it is increased only to -0.083 ± 0.084; i.e., is still no greater than its probable error and therefore of no value. A more complete correction for the possible disturbing influence of temperature is obtained by applying partial correlation to the entire series of data according to the formula:

$$r_{12.3} = \frac{r_{12} - r_{13} \cdot r_{23}}{1 - r_{13}^2 \cdot 1 - r_{23}^2}$$

where r_{12} = correlation between weight and oxygen per unit weight
r_{13} = correlation between temperature and oxygen per unit weight
r_{23} = correlation between temperature and weight.

This gives a value of -0.097, which again is no indication of well-defined relationship.

The entire data were then recalculated on the basis of weight to the two-

thirds power; which, as is well known, is more nearly proportional to body surface; with, if anything, greater dispersion; the results being:

Range = 4.5 to 14.0 cc. per kgm.$^{\frac{2}{3}}$ per minute.
Standard deviation = 1.86
Coefficient of variation = 20.1

It seems impossible, therefore, to escape the unexpected conclusion that these anesthetized animals do not obey the surface-area rule; and that although their metabolic rate is not very constant per unit of weight it is no more constant when reduced to a unit proportional to body surface.

5. Comparison with normal, unanesthetized cats. Oxygen consumption. Data for this use are not too abundant; some may be found in the publications of Aub, Bright and Uridil (1922) and of Hunt and Bright (1926); our own are as follows and consist of 32 observations on 7 normal male cats. With two of these the respiratory metabolism was determined with

TABLE 2

Data of normal unanesthetized cats

FUNCTION	NUMBER OF DETERMINATIONS	RANGE	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
Body weight (kgm.)	7	2.5–3.87	3.13		
Oxygen consumption (cc./kgm./min.)	32	4.3–11.8	6.74	2.07	30.7
Carbon dioxide output (cc./kgm./min.)	32	3.7–9.6	5.81	1.40	24.1
Respiratory quotient	32	0.62–0.88	0.746	0.067	8.98
	(8)	(0.62–0.70)			
Alcohol checks	14	0.644–0.665	0.657		

a Benedict type of closed-circuit apparatus; with the other five Haldane's open-circuit method was used. The results are shown in table 2.

Comparing the rates of oxygen utilization of our normal (6.74 cc. per kilo per minute) and anesthetized (6.43 cc. per kilo per minute) cats there is an apparent decrease due to the anesthetic of 0.31 cc. per kilo per minute, or 4.6 per cent. It is necessary, however, to make allowance for the difference of body temperature. As mentioned in a previous section, calculating on the basis of the temperature effect observed in these anesthetized animals, at normal (cat) temperature of 38.7°C. their average rate of oxygen utilization could be expected to be 7.18 cc. per kilo per minute; this is 0.44 cc. per kilo per minute, or 6.5 per cent above the observed average for the normal, unanesthetized animals of 6.74 cc. per kilo per minute.

We thus arrive independently at the conclusion previously reached by Aub, Bright and Forman, and by Hunt and Bright: viz., metabolic rate is increased under anesthesia; the increase of 6.5 per cent under chloralose

is a less abnormality than the elevation of "over 10 per cent" reported by Hunt and Bright for amytal, or the 15 per cent increase found by Aub and his co-workers for urethane. By the latter this was attributed to stimulated activity of the adrenals; Hunt and Bright considered this less probable under amytal; only further work can determine whether the same cause is responsible in the three different instances and exactly what it is.

It may be pointed out, in conclusion, that the coefficients of variability for oxygen consumption (30.7), carbon dioxide output (24.1) and respiratory quotient (8.98) are of the same order of magnitude, but even greater, in these normal animals than they are in the anesthetized condition. This is not surprising in view of the difficulty of obtaining strictly basal states in an active, normal cat; it might have been expected, however, that a greater uniformity would have obtained in the complete quiesence of anesthesia. At least, however, the variability under chloralose cannot be attributed in any large measure to the action of the anesthetic; but rather to its inability to suppress in any considerable degree normal individualities of metabolism.

The respiratory quotient. Aside from its quantitative effect it might appear from the low quotients observed in these anesthetized animals that chloralose produced qualitative changes in metabolism. It will be recalled that the range of the 84 determinations was from 0.64 to 0.86, and the average, 0.745; with 12, or 14 per cent, of the values below 0.70. Referring to the data for normal cats (table 2), however, it will be seen that almost precisely the same condition obtained with them: the 32 determinations had almost exactly the same range (0.62 to 0.88) and average (0.746); and 8, or 25 per cent, were below 0.70. The range (0.644 to 0.665) and average (0.657) of the alcohol checks run concurrently with these determinations and inspire confidence that they are probably not too much in error. It would appear, therefore, that chloralose has no specific effect on qualitative metabolism; nor does the cat seem more prone to development of low quotients in the post-absorptive state than the rabbit (Lee, 1939).

SUMMARY

Data are given describing the range, mean, standard deviation, coefficient of variation, and interrelationships, as established by the coefficient of correlation, for: blood pressure and heart rate, blood sugar and lactic acid, pulmonary minute volume, carbon dioxide output, oxygen consumption and respiratory quotient of 84 cats under chloralose anesthesia; and, in addition, for comparison, 32 determinations of: oxygen consumption, carbon dioxide output and respiratory quotient of 7 normal unanesthetized cats.

During the second to fourth hour of anesthesia all of these physiological factors are practically unchanging over short experimental periods of 15 to 20 minutes' duration.

Inter-individual variability of all functions (range and coefficient of variation) is large; the variations appear, however, to be normally interrelated (coefficients of correlation), indicating that chloralose produces no grossly abnormal physiological disturbance; in particular the respiratory quotient appears unaffected.

Oxygen consumption of the anesthetized animals is scrutinized in more detail and is shown: 1, not to be affected by duration of anesthesia within the time limits employed here; 2, nor to owe its variability per unit of body weight to inverse relationship with this factor; 3, to vary according to sex, season and body temperature; and 4, to be increased approximately 7 per cent by the anesthetic.

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THE ELECTRICAL EXCITABILITY OF THE SUPERIOR CERVICAL GANGLION OF THE CAT

G. H. ACHESON AND F. A. SIMEONE

From the Department of Physiology in the Harvard Medical School

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While much precise information has accumulated concerning the excitability of nerve axons, little is known about this aspect of the other parts of the neuron, namely, the cell body and the dendrites. In the oculomotor neurons Lorente de Nó (1935) demonstrated that the cell body (including the dendrites) is electrically excitable. The present work was intended to reveal whether or not the same is true of the postganglionic sympathetic neurons whose cell bodies lie in the superior cervical ganglion.

The method employed was the comparison of excitability curves of the pre- and postganglionic nerves with similar curves taken with the stimulating electrodes in a favorable position for the stimulation of the ganglion cells. The curves studied were either voltage-capacity or voltage-response curves. For multifibered nerves these curves are commonly found to be continuous.

Lucas (1907a, b) was the first to point out that a discontinuity in the strength-duration curve might indicate that the organ contained two or more groups of excitable elements with different characteristics of excitation. By comparing the curves for nerve and for nerve-free muscle with that for innervated muscle, he was able to identify in the complex curves of the latter the components contributed by nerve and by muscle. Hill (1936) and Maltesos and Schneider (1938) found breaks in the strength-duration curves of nerve trunks; and these cases they attributed to a grouping of nerve fibers about two or more levels of excitability much as Lucas had found for nerve and muscle.

Voltage-response curves describe the distribution of thresholds of electrical excitability of the elements in the field of stimulation in terms of a response which is cumulative as the voltage increases above the lowest threshold. Here breaks in the smoothness of the curve would denote grouping of relatively more elements at certain thresholds than are present at others.

The superior cervical ganglion resembles the innervated muscle in Lucas' experiment. It includes both preganglionic axons, corresponding to Lucas' "nerve," and postganglionic neurons, corresponding to Lucas'

"muscle." The excitability curves of the preganglionic axons can be tested caudad along the cervical sympathetic trunk, and those of the postganglionic axons can be tested cephalad to the ganglion. The excitability curve of the ganglion, like that of Lucas' innervated muscle, might be expected to contain a break, marking the crossing of the curves of these two components of the ganglion.

The ganglion may, however, be simplified by section and degeneration of the preganglionic axons. It now contains only the various parts of the postganglionic neurons (dendrites, cell bodies and beginnings of axons). Any difference between the excitability curves of the ganglion and those of the postganglionic nerve can be attributed to the special features of the ganglion. In particular, given smooth curves for the postganglionic nerves, a break in the excitability curves of the ganglion suggests that the ganglion contains two groups of electrically excitable elements, one of them the postganglionic axons and the other, therefore, one of the other parts of the postganglionic neuron, either the ganglion cells or their dendrites.

METHOD. Cats were used under dial anesthesia (Ciba, 0.8 cc. per kgm.). "Denervated ganglia" were those whose preganglionic nerve trunk had been sectioned aseptically 1 to 4 weeks previously. The complete degeneration of the nerve was checked in the acute experiments by failure of response of the iris and nictitating membrane on tetanic stimulation of the remains of the nerve.

Care was taken to preserve the integrity of the ganglion during operative procedures. Nerves IX, X, XI and XII were sectioned centrally and peripherally to the region of the ganglion to avoid the effects of spread of stimulating current. Pairs of chlorided silver electrodes, each less than 0.5 mm. in diameter, were placed at the cephalic and caudal tips of the spindle-shaped ganglion. The caudal pair served for preganglionic, the cephalic pair for postganglionic stimulation, and one of each for stimulation of the ganglion itself. The electrodes of the pre- and postganglionic pairs were separated by 2 to 5 mm., while the distance between the electrodes adjacent to the ganglion was 6 to 15 mm.

Condenser discharges of known capacity and voltage were used for stimulations, the cathode being cephalad. In some animals a constant frequency (1 to 5 per second in different experiments) was applied for a definite period or until the response had reached a plateau. With this mode of stimulation isotonic contractions of the nictitating membrane were recorded on a smoked drum by a light lever giving a magnification of about 20 times. In other animals single shocks were applied and the excursions of a beam of light were observed as reflected to a millimeter ruler from the mirror of a frictionless torsion spring myograph registering tension of the nictitating membrane. The greatest excursion of the free

border of the nictitating membrane in the latter case was less than 2 mm., and the magnification by the beam mechanism was about 60 times. In any experiment the interval between stimulations was fairly constant.

Responses of the nictitating membrane were recorded for different voltages at the same capacity (voltage-response curves), or voltage-capacity curves were built for a response of given height, usually 30 to 60 per cent of maximal (Rosenblueth and Rioch, 1933). Double logarithmic plotting was adopted for the latter. For voltage-response curves, the following procedure was adopted in order to check their accuracy: first, a few random voltages were applied in order to estimate the range of the curve; next, a series of 10 or more points was taken with increasing voltages from a very small to an almost maximal response; then, a second more detailed series was taken from higher to lower voltages. Sometimes three or four such series were taken in alternating direction, up and down the voltage scale.

RESULTS. A. *Pre- and postganglionic nerves.* Some observers (Rosenblueth and Rioch, 1933; Knoeffel and Davis, 1933) have reported smooth voltage-response and voltage-capacity curves for the pre- and postganglionic nerves. Maltesos and Schneider (1938), however, report that in some of their experiments there were breaks in the voltage-capacity curves of the cat's cervical sympathetic nerve. The observations of the latter workers were not confirmed in the present work, since all curves for the nerve-trunks were smooth (fig. 1), even in animals whose ganglion curves (sections B and C) showed breaks.

B. *Normal ganglion.* In 11 normal animals reliable ganglion curves were built. While 2 of these showed smooth voltage-response curves similar to those of the pre- and postganglionic nerves (section A), in the other 9 the voltage-response curves were complex, as shown by breaks. Two of these curves had one break, and each of the remaining 7 showed two breaks (fig. 2). Two voltage-capacity curves were built; of these 1 showed one break, the other, two breaks (fig. 3).

C. *Denervated ganglion.* In 8 animals reliable curves were built for denervated ganglia. Of 7 voltage-response curves, 3 had one break and 4 had two breaks (fig. 4). Of 2 voltage-capacity curves, 1 had one break and 1 had two breaks.

D. *Ganglion destroyed.* After the building of ganglion curves showing breaks (sections B and C), the ganglion between the electrodes was crushed (in 1 animal) or extirpated (in another) and curves were built, using the same electrodes formerly employed for ganglion curves. The voltage-response curves were now smooth.

E. *Variability of response.* Spontaneous variations of the length or tension of the nictitating membrane were frequently encountered in animals with denervated ganglia. The eyeball was removed and the striated

muscles of the orbit were cut to decrease these changes. During the construction of curves reported above spontaneous activity was minimal.

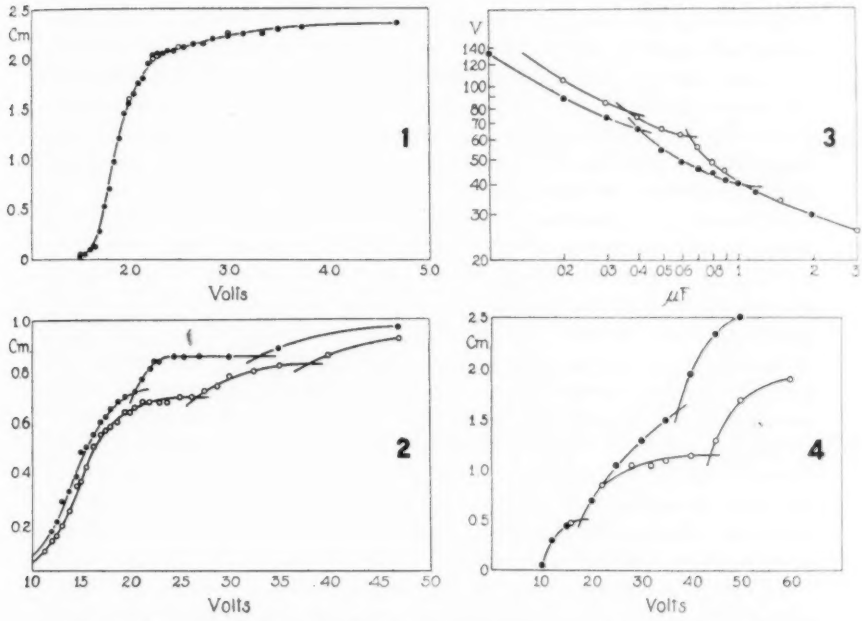

Fig. 1. Cat under dial anesthesia. Voltage-response curve of preganglionic nerve. Each point represents a response of the nictitating membrane to 10 condenser discharges (0.1 μ F) at 4 per sec. Ordinates: volts. Abscissae: response in centimeters.

Fig. 2. Voltage-response curves of superior cervical ganglion. Ten condenser discharges (0.1 μ F) at 4 per sec. Dots: earlier curve, ascending the voltage scale. Circles: later curve, ascending the voltage scale.

Fig. 3. Voltage-capacity curves of the superior cervical ganglion for 2-cm. responses of the nictitating membrane to condenser discharges (5 per sec. for 30 sec.). Dots: earlier points. Circles: later points. Ordinates: volts. Abscissae: microfarads. Double logarithmic plotting.

Fig. 4. Voltage-response curves of denervated superior cervical ganglion. Single condenser discharges (0.02 μ F). Dots: descending the voltage scale. Circles: ascending the voltage scale.

It was usually found in building ganglion curves that after one curve had been completed a repetition of the procedure yielded a curve similar in shape, but shifted along the voltage scale. Figure 4 illustrates this change; first a descending and then an ascending series of voltages were

applied. This shift represented in most cases a decrease of excitability but in some, an increase.

Discussion. The essential fact reported is that excitability curves taken with the electrodes favorably situated for the stimulation of ganglion cells have a more complex configuration than do the curves of the pre- and postganglionic nerves, in that the ganglion curves show breaks while the latter are smooth. This is true even when the ganglion is simplified by removal of one set of its elements, the preganglionic axons. These facts indicate that the neurons connecting with the nictitating membrane have different characteristics of excitation when the ganglion is stimulated from those when the postganglionic axons are stimulated.

Maltesos and Schneider (1938) argued from their finding of breaks in the voltage-capacity curves of preganglionic nerve trunks that the axons serving the nictitating membrane are grouped about two or more levels of excitability. The same explanation might be applied to the breaks in ganglion curves reported above, namely, a grouping about several levels of excitability of the ganglion cells serving the nictitating membrane. In the present work, however, the smooth curves of the postganglionic nerve reveal that the axons are not so grouped, even in animals showing breaks in ganglion curves. This explanation can therefore be applied to the breaks in ganglion curves only if the ganglionic parts of the neurons have different characteristics of excitation from those of the axons. As was concluded above, this condition itself accounts for the breaks when the postganglionic curves are smooth.

The most likely characteristics of the ganglion to account for the singularities of its excitability curve are the anatomical features of the neurons in the ganglion which are absent in the postganglionic trunk, namely, the cell body and the dendrites. The data suggest the presence in the denervated ganglion of three groups of excitable structures, each represented by a segment of the doubly-broken curve (fig. 4). One of these components undoubtedly represents the postganglionic axons. This leaves two components of the curve to represent two other groups of excitable structures in the strictly ganglionic portion of the neuron, i.e., that part which includes the cell body and the dendrites. Assignment of a segment each to these two structures would be mere speculation. The present data do, however, lead us to the conclusions that some part of this strictly ganglionic portion is electrically excitable and that the characteristics of excitation of this part differ from those of the postganglionic axon.

SUMMARY

1. Voltage-capacity and voltage-response curves were built for the superior cervical ganglion of the cat and for its pre- and postganglionic nerves. Responses of the nictitating membrane were used as an indicator of the number of elements excited.

2. In confirmation of the work of Rosenblueth and Rioch (1933) and of Knoeffel and Davis (1933), but at variance with the results of Maltesos and Schneider (1938), these curves for pre- and postganglionic nerve trunks are found to be smooth (fig. 1, p. 575).

3. Curves built with the electrodes at the cephalic and caudal tips of the ganglion present one or two breaks. This is true both of the normal ganglion (figs. 2 and 3) and of the ganglion which has been denervated by previous section and degeneration of its preganglionic fibers (fig. 4; p. 575).

4. It is concluded that the ganglionic part of the postganglionic neuron, namely, the part which includes cell body and dendrites, is electrically excitable and has different characteristics of excitation from those of the postganglionic axon (p. 576).

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ELIMINATION OF SODIUM IN PANCREATIC JUICE AS MEASURED BY RADIOACTIVE SODIUM[1]

M. LAURENCE MONTGOMERY, G. E. SHELINE AND I. L. CHAIKOFF

From the Division of Surgery (San Francisco) and the Division of Physiology (Berkeley), University of California Medical School

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Recent developments in the use of radioactive isotopes have clearly established their value as tracers in the study of movements of elements and compounds in the animal body. The application of tagged elements to the study of the activities of the glands of the gastrointestinal tract would appear to offer certain advantages, since the high degree of radioactivity which may be induced in a small amount of material makes possible the introduction of such minute amounts of labeled salts that they need not alter the normal physiological state of the animal. In addition, the sensitivity of the method available for the detection of radioactivity is such that the labeled salt may be accurately followed, even when only a small fraction of it makes its appearance. The present investigation deals with the secretion of labeled sodium by the pancreas.

EXPERIMENTAL. *Preparation and care of animals with pancreatic fistulae.* Large dogs were used throughout this study. The upper duct of the pancreas was ligated and the lower duct cannulated by a modification of the procedure of Elman and McCaughan (1). A soft rubber tube 7 inches long was used intra-abdominally, while a heavier walled tube 6 to 7 inches long was brought out through a left-upper rectus stab wound. A small tin foil-coated cellophane disc was placed over the glass connector between the two rubber tubes and acted as a stop to prevent extrusion of the rubber tubes through the stab wound. The soft rubber tubing, the cellophane disc, and the first portion of the heavy rubber tubing were wrapped in the distal 1 inch of the omental margin, beginning at a point 3 inches from the pylorus and extending to the neighborhood of the spleen. This produced a gentle semicircular curve of the tubing from the cannula to the stab wound, a procedure that tends to reduce the danger of kinking. The omentum also served to seal the cannula-duct area and to plug the peritubular region at the stab wound, so that no infection could be introduced

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into the general abdominal cavity. The capacity of the tubular system was approximately 2 cc. For the purpose of collecting the pancreatic juice at the times when the animal was not under observation, a sterile rubber bag was attached to the tubing.

The fistulae dogs were maintained in good condition by replacement therapy and an adequate diet. Each animal received daily 1 to 2 liters of Ringer's solution intravenously to insure an adequate supply of salts and liquid. The dogs were fed a basic diet of lean meat and beef lungs. Vitamin supplements were added: A and D in cod liver oil; the B complex in a concentrate obtained from rice bran. Each animal also received 100 grams or more of raw pancreas daily. It has been repeatedly observed here that the daily feeding of the raw glandular tissue provides for a satisfactory nutritional state in dogs with pancreatic fistulae.

Collection of pancreatic juice. Dogs that possessed a satisfactory secretory mechanism on the day of the experiment were accepted as adequate preparations. Stimulation to secretion was produced by the ingestion of 200 grams or more of lean meat 0.5 to 2 hours before the commencement of the collections and 100 grams of meat at intervals thereafter. The dog was laid on its left side with the rubber tubing hanging over the edge of the table. An initial control collection was made 10 to 20 minutes before the introduction of the radioactive sodium into the vein. Then 2 cc. were collected and discarded as representing the residual content of the tubal system. The juice was collected in graduated centrifuge tubes, measured and sampled. Collections were made at approximately 3, 7, 10, 15, 20, 25, 30, 45 and 60 minutes, and at 0.5-hour intervals thereafter for 8 to 9 hours. The animal was then returned to its cage for the night with the rubber bag attached. The following morning the juice that had accumulated during the night was measured and sampled. A final sample was then obtained by collecting the secretion produced during the next 10 to 15 minutes.

Collection and preparation of blood serum. Blood samples were removed from the jugular or leg veins at intervals during the course of the experiment. In no instance was blood taken from the same vessel in which the labeled sodium was injected. Three to 6 cc. of blood were removed at a time, allowed to clot firmly, and then centrifuged at 3000 RPM for 15 minutes. Serum was separated as soon as possible after the removal of blood from the animal.

Preparation of labeled sodium. A layer of metallic sodium was spread over the surface of a copper plate that had perviously been cleaned with concentrated nitric acid. This target was then subjected to a deuteron bombardment in the Berkeley cyclotron. A combination of scraping and washing with 50 per cent alcohol was employed to remove the bombarded sodium from the target. The first step in the purification was to acidify

the solution with HCl and filter out any material which did not dissolve. The solution was evaporated to dryness on a steam bath and then heated with an open flame to insure removal of all traces of alcohol and HCl. To eliminate copper and other heavy metals which might be present, the residue was dissolved in distilled water and the solution saturated with hydrogen sulfide. The sulfide precipitate was filtered out and the excess hydrogen sulfide washed from the solution by bubbling a stream of air through the filtrate. After determining the weight of NaCl present, the salt was dissolved and made up to a 1 per cent solution. A small portion of this solution was evaporated on blotter paper measuring 3.5 x 6 cm. The blotter was then wrapped and sealed in cellophane and an assay of its radioactivity made by a Geiger-Müller counter, which had previously been standardized against a sample of uranium so that the number of emanations detected by the counter per unit of time could be converted into millicuries.

Radioactive sodium was introduced into the hind-leg vein of a dog, and at given intervals thereafter, as previously noted, the secreted juice was measured and blood taken for serum analysis. Samples of the juice and serum were then prepared and tested by the methods described below.

Measurement of radioactivity. Five-tenths or 1.0 cc. samples of pancreatic juice were carefully measured and deposited dropwise upon a blotter measuring 3.5 x 6 cm., which was suspended over an electric hot plate. When thoroughly dry, the blotter was wrapped and sealed in cellophane. Five-tenths cubic centimeter of serum was transferred to blotters and treated in a similar manner. The activities of the blotters were measured with the Geiger-Müller counter, as previously described. Great care was taken to insure uniformity in the mounting of the samples. The sensitivity of the counter was standardized by means of a thorium source before and after each determination. Duplicate samples of serum and pancreatic juice were taken for analysis, and in all cases the results recorded represent the averages of two closely agreeing values.

Results. Observations were made on 9 dogs with pancreatic fistulae; the results obtained were in essential agreement. The excretion of labeled sodium was followed for 22 to 24 hours after the intravenous injection of the radioactive salt. The quantity of sodium chloride injected varied from 40 ml. of an isotonic solution containing a total of 0.04 millicurie of radioactivity to 10 ml. containing 0.96 millicurie. Differences attributable to these variations were not observed. A typical experiment in which 40 ml. of an isotonic solution of sodium chloride containing 0.04 millicurie was injected into a dog weighing 22 kgm. is recorded in figure 1.

The promptness of appearance of administered sodium in the external secretion of the pancreas was noted in all dogs. In 4 animals radioactivity was found in the first sample obtained at the end of 3 minutes. In the

other animals the first samples of pancreatic juice were not taken until intervals of 5, 6 or 10 minutes had elapsed; in all cases labeled sodium was

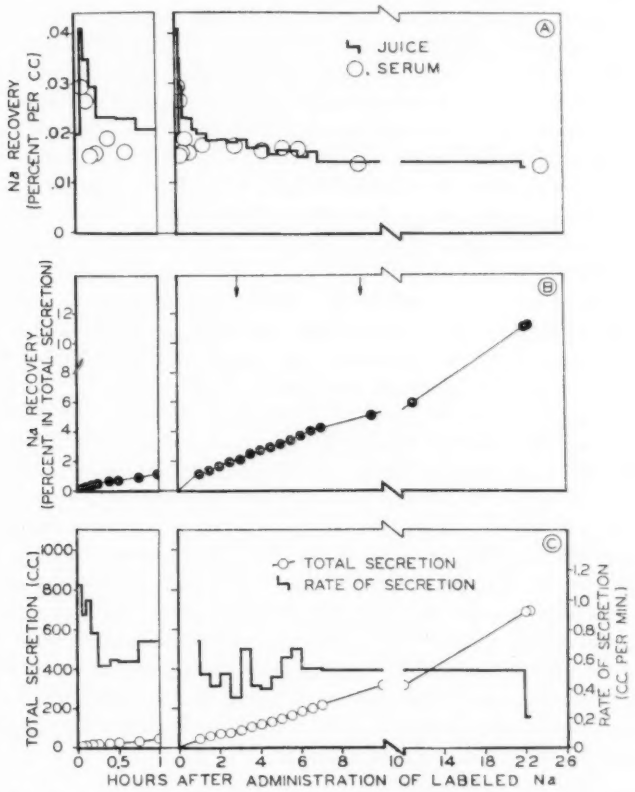

Fig. 1. The secretion of radioactive sodium in pancreatic juice. Dog III, weight 22 kgm. Forty cubic centimeters of 0.9 per cent solution of sodium chloride containing radioactive sodium were injected intravenously at the O interval. In A the concentration of labeled sodium in pancreatic juice and serum is expressed as the percentage of the administered labeled sodium recovered per cubic centimeter. In B the total recovery of labeled sodium is shown and expressed as percentage of the administered labeled sodium. The arrows show the times at which meat was fed to stimulate pancreatic secretion. This animal also received 200 grams of lean meat one hour before the O interval. In C the rate of secretion as well as the total secretion is recorded.

already present. Although the activities of the initial samples were usually low, the highest concentrations of labeled sodium occurred during the first hour. In dog I, labeled sodium was found in increasing amounts in

all samples of pancreatic juice removed during the first 15 minutes after the administration of the radioactive salt, the maximum concentration appearing in the sample obtained between 10 and 15 minutes. In dog II, the highest concentrations were found in the samples obtained after the first 10 minutes, and the content of labeled sodium in the pancreatic juice remained high for the next 50 minutes. The maximum for dog III occurred between 3 and 6 minutes after the injection. The quicker response in this animal is probably the result of the rate of secretion, for it secreted pancreatic juice more rapidly than either dog I or dog II during the early intervals after the administration of labeled sodium.

The similarity in the concentration of labeled sodium in serum and in pancreatic juice is indeed striking. Thus in dog I the sample of serum obtained at the 2-hour interval contained 0.017 per cent of the administered labeled sodium per cubic centimeter, whereas the sample of pancreatic juice removed between the 2 and 2.5-hour interval contained 0.018 per cent per cubic centimeter. At the same time intervals, the serum and pancreatic juice of dog II contained respectively 0.024 and 0.025 per cent of the administered radioactive sodium per cubic centimeter. The sample of pancreatic juice obtained from dog III (fig. 1) between 2 and 2.5 hours contained 0.019 per cent per cubic centimeter, the serum at the 3-hour interval 0.018 per cent of the administered radioactive salt per cubic centimeter. Moreover, the higher concentrations of labeled sodium found during the early intervals parallel the higher concentrations of radioactive sodium present in the serum at this time. It should be noted, however, that in the early periods the concentrations of labeled sodium appear to be somewhat lower in the serum than in the pancreatic secretion. This relation between the levels of labeled sodium in serum and juice is in keeping with previous observations of Gamble and McIver (2) and of others (3, 4), who found that pancreatic juice contains fixed base in concentrations approximating those found in the plasma.

The radioactive sodium used in this investigation was supplied by members of the Radiation Laboratory under the direction of Prof. E. O. Lawrence, to whom our thanks are due.

SUMMARY

1. The elimination of sodium in the external secretion of the pancreas was investigated in dogs provided with pancreatic fistulae with the aid of the radioactive isotope of sodium.

2. Labeled sodium made its appearance in samples of pancreatic juice obtained as early as the first 3 minutes after the intravenous injection of the radioactive salt. Maximum concentrations were found within 15 minutes.

3. Except in the early periods, the concentrations of labeled sodium in the pancreatic juice closely follow those observed in the serum.

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UTERINE RESPIRATION, CYTOCHROME OXIDASE AND COPPER[1]

MARK GRAUBARD

From the Biological Laboratories, Clark University, Worcester, Massachusetts

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The suspicion that cytochrome oxidase may contain copper as its active agent grew with the finding of this metal in tyrosinase and polyphenolase (1, 4, 5, 7). The type of substrate and mode of action characterizing these three enzymes are sufficiently similar (2) to warrant the expectation.

To obtain conclusive evidence for the presence of copper in cytochrome oxidase proved difficult because of the insolubility of the oxidase in any medium tested so far. The quantities present are of such concentrations that the suspended state of the enzyme cannot permit even approximate determinations of any reliability.

Obviously such determinations could be made if the enzyme were obtained in soluble form and subsequently concentrated. This has failed and experiments were therefore undertaken to prove the presence of copper in the oxidase by indirect methods.

EXPERIMENTAL. As the source of our cytochrome oxidase we used uteri of rabbits. This tissue contains very little cytochrome but ample oxidase. The oxidase is denatured by water so that unlike beef heart, the ground uterine tissue cannot be washed to remove water soluble material and extraction is made with M/15 phosphate buffer at pH 7.3.

The tissue is ground with sand, the mixture centrifuged and the supernatant used. Measurements of enzyme quantities are determined in Barcroft-Warburg respirometers using p-phenylenediamine as substrate with added excess cytochrome prepared from beef heart. The temperature of the thermostat is 37.50 ± 0.05°C. and the pH of the experimental mixture 7.30.

To begin with, it should be noted that whether the interesting suggestion of Stern (3) with regard to the micelle nature of cytochrome oxidase is fully verified or not, it seems clear that the uterine tissue has only a limited amount of enzyme in the same way as any soluble compound is distributed in any other tissue. Thus, after the oxidase is extracted once

[1] Aided by a grant to Dr. G. Pincus from the National Research Council Committee for Problems of Sex and the Works Progress Administration (Project no. 665-14-3-726).

with phosphate buffer, much tissue remains in the centrifugate but no more enzyme can be obtained from it on further grinding.

Uterine tissue can be made to yield a soluble cytochrome oxidase which, however, is not stable. An enzyme preparation containing 1.5 to 2.0 units per cubic centimeter and appearing homogeneous though slightly opalescent will, on being passed through a bacterial Seitz filter yield a water clear solution. If tested at once the filtrate can be shown to contain up to 0.5 unit per cubic centimeter. It should be stated however that only four such positive results were obtained out of a total of nine trials. The other five yielded no enzyme in the filtrate. Within several hours the activity vanishes and simultaneously a turbidity appears in the solution. It may be assumed that a small fraction of the oxidase consists of sufficiently small particles to form a true solution but these are not stable. They reform the larger particles which do not retain the previous activity of their components. Concentrating and purifying this soluble fraction proved impossible and the extreme dilution precluded any estimation of copper.

Experiments were therefore performed on the effect of copper inhibitors on the oxidation of p-phenylenediamine by copper and by cytochrome oxidase. Several copper inhibiting substances were selected, namely, potassium cyanide, diethyl-dithio carbamate, salicylaldoxime, potassium ferrocyanide, thiourea, hydroxyquinoline and potassium xanthogenate. Each of these was checked in the system-copper sulphate, phosphate buffer, p-phenylenediamine and found to inhibit the oxygen uptake.

It was found that thiourea had a relatively weak inhibiting action on cytochrome oxidase requiring 0.3M solution to reduce the O_2 uptake to 25 per cent, while hydroxyquinoline and potassium xanthogenate, though definitely showing inhibition are however involved in complicating secondary reactions. Results with the first five copper inhibiting substances listed above are recorded in figure 1. With inorganic copper the inhibiting action of these compounds is more or less stoichiometric. This evidence can therefore be taken to indicate that copper is in all likelihood the active metal component in the system cytochrome oxidase and cytochrome.

That it is the cytochrome oxidase that is involved in the inhibition is suggested by the following experiments. To a series of respirometer vessels all containing p-phenylenediamine, buffer, cytochrome and oxidase, any one of the copper inhibitors cited in figure 1, except thiourea, is added in amounts just sufficient to bring about complete inhibition. Fresh cytochrome is then added to one vessel, more enzyme to another, more substrate to a third and buffer to a fourth, the latter to act as control. It is then observed that upon addition of cytochrome no renewed oxygen uptake occurs. Upon addition of new oxidase the uptake is proportional

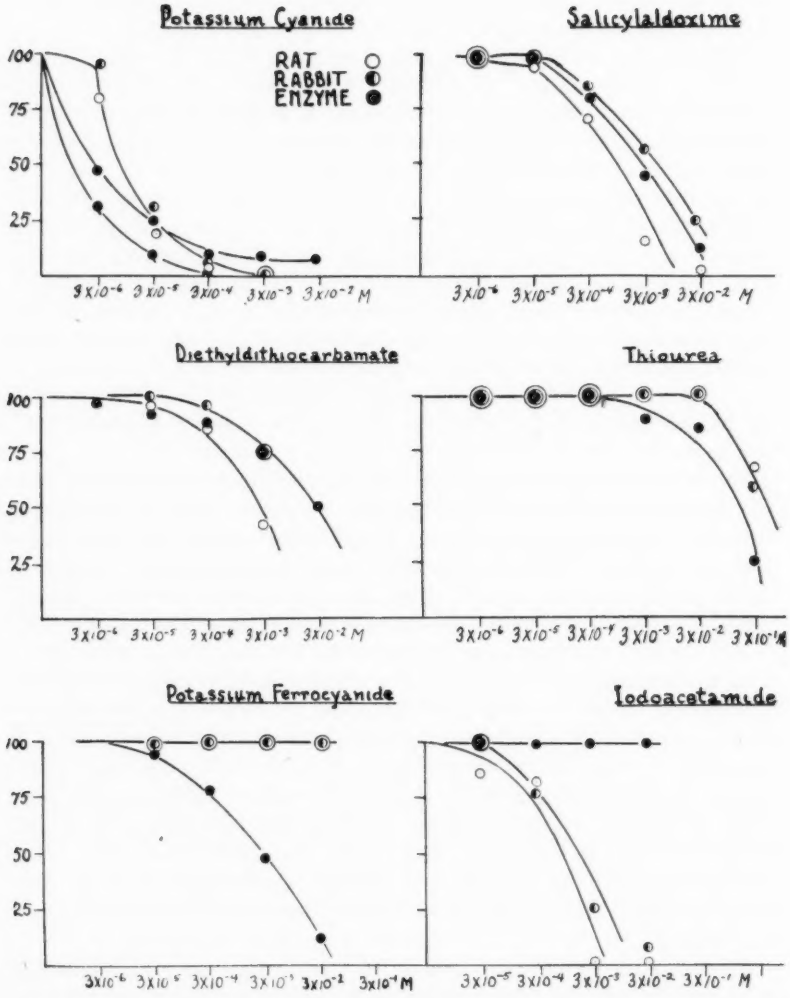

Fig. 1. Curves showing inhibition of cytochrome oxidase extracted from rabbit uteri by five copper inhibitors and failure of iodoacetamide to inhibit it. These curves are to be compared with curves showing inhibition of respiration by rabbit and rat uteri by the same substances. Pregnant and non-pregnant rabbit uteri, endometrium and whole uterus give similar results with regard to inhibition.

Abscissae = concentration of inhibiting substance in experimental vessel.

Ordinates = Q_{O_2} expressed as percentage of normal uptake.

to the amount of enzyme added. It should be noted however that some renewed oxygen uptake occurs as well upon addition of more substrate though such rate is never more than about half the rate obtained after addition of fresh enzyme. This evidence seems to indicate that the copper inhibitors do not affect the cytochrome but primarily the enzyme or enzyme substrate combination.

Experiments were further undertaken to study the effect of these five copper inhibiting compounds upon the total respiration of rabbit and rat uterine tissues. Whole and endometrial tissues of rabbit uteri of non-pregnant and three day pregnant animals were used as described previously (3). The respiration was observed in serum or buffer media. As reported previously no significant differences were observed in these two media. The respiration is calculated on a basis of cubic millimeter of oxygen per hour per miligram dry weight of whole uterus or endometrial tissue. The results are shown in figure 1. It should be noted that the inhibition of various concentrations of diethyl-dithio carbamate could not be completely determined because when concentrations higher than 0.03M are employed there is an evolution of gas and no measurements of oxygen uptake are possible.

Similar experiments were performed with whole rat uteri. These were obtained from 25 to 27 day old animals either normal or injected with pituitary extracts for bioassay. The uteri are placed in respirometer vessels and the rate of oxygen uptake determined per milligram dry weight as described elsewhere (6). Figure 1 indicates that the two types of uteri behave much the same with regard to copper inhibitors.

It should be observed that with potassium ferrocyanide no inhibition of uterine respiration is obtained. This is the only copper inhibitor which fails to inhibit tissue respiration. It may be of interest to recall that many plant and animal tissues do contain an enzyme which oxidizes ferrocyanide to ferricyanide (2). This phenomenon may have some bearing on the exceptional behavior of potassium ferrocyanide. Also in the presence of potassium ferrocyanide the oxygen uptake is slightly higher than in the control.

Discussion. The method employed here in testing the presence of copper in cytochrome oxidase is obviously indirect. The fact that practically all known copper inhibitors inhibit the action of cytochrome oxidase seems to make difficult any other conclusion but that the above oxidase contains copper as its active metal.

A comparison of the inhibition curves of rat and rabbit uteri brings out the close resemblance in the relationship of concentration to inhibition in both enzyme and respiring tissue. In both cyanide seems to inactivate more directly, while both enzyme and tissue show strong buffering power toward the other copper poisons.

It seems reasonable to conclude that cytochrome oxidase is responsible for the total respiration of rabbit and rat uterine tissues. Yet that must not be taken to mean that the respiration of these tissues is controlled exclusively by the cytochrome-cytochrome oxidase system. The curves for the inhibition of uterine respiration by iodoacetamide and its failure to affect the enzyme given in figure 1 prove this point (6, 8). The iodoacetamide is almost as effective an inhibitor of tissue respiration as is cyanide, yet it exerts no influence whatever on the action of the enzyme. This implies that several fundamental reactions may be involved in the control of oxygen uptake by respiring tissue and that such uptake may be blocked correspondingly by different agents.

SUMMARY

1. A water soluble cytochrome oxidase is reported and its transient nature and rapid inactivation described.

2. The inhibition of cytochrome oxidase by substances which inhibit copper catalysis was studied. Evidence is presented which indicates that copper is the active metal of cytochrome oxidase.

3. The rates of oxygen uptake of uteri of rats and of pregnant and non-pregnant rabbits are affected by copper inhibitors in the same manner as is cytochrome oxidase. Hence it is concluded that cytochrome oxidase is directly responsible for the overall respiration of uterine tissues though other substances or reactions may be so linked with it as to be equally determinant.

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THE EFFECT OF ADRENALECTOMY ON THE HISTAMINE CONTENT OF THE TISSUES OF THE RAT

BRAM ROSE[1] AND J. S. L. BROWNE

From the McGill University Clinic, Royal Victoria Hospital, Montreal, Canada

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It has been shown by many investigators that there is a relationship between the adrenal gland and the metabolism of histamine. Following the removal of the adrenal gland in the rat there occurs a decrease in resistance to histamine (Wyman, 1928) which is associated with a marked loss in the ability of the animal to destroy histamine (Rose and Browne, 1938) and a decrease in the histaminase content of lung tissue (Rose and Karady, 1939). It has also been shown that the ability of the adrenalectomized rat to inactivate histamine, and that the decrease in histaminase content of lung tissue following adrenalectomy can be restored to normal by the administration of adequate amounts of adreno-cortical substances (Rose, 1939; Karady, Rose and Browne, 1940).

In view of these findings it was thought that a study of the histamine content of the tissues of the rat before and after adrenalectomy might be of interest.

METHODS. Rats of a hooded strain weighing from 160 to 200 grams were used. The animals were maintained on purina chow and given water to drink. Three groups of animals were used. The first group consisted of 12 animals which served as controls. Since it has previously been shown that the decrease in resistance to histamine in rats following adrenalectomy and maintained on normal saline is fully established by the seventh to eighth day following removal of the adrenal glands (Gottesman and Perla, 1931), a second group of twelve animals was adrenalectomized, maintained on purina and given 0.85 per cent saline to drink for eleven days in order to be certain that the decrease in resistance was fully established. The animals were used on the twelfth day after adrenalectomy. A third group of six animals was adrenalectomized maintained on purina and given 0.85 per cent saline to drink for seven days. The saline was then replaced by water for an additional four days. These animals were quite healthy while they received saline to drink, but they rapidly manifested marked signs of adrenal insufficiency such as weakness, diarrhea and loss of weight when water was substituted. They were killed on the twelfth

[1] Aided by a grant from the Banting Research Foundation.

day after adrenalectomy and the histamine content of the tissues determined. It was noted at the time the tissues were removed that hemorrhagic spots and ulcerations were present in the stomach and small intestine.

Examination of the blood and tissues for histamine was carried out as follows. The animals in each group were anesthetized with ether, the abdomen opened, and a specimen of blood removed from the inferior vena cava. The tissues were then removed and washed free of blood in normal saline. Stomach and small intestine were opened and washed free of their contents. The excess moisture was removed by placing the tissues on large sheets of filter paper. The tissues were then placed in previously weighed flasks containing 10 per cent hydrochloric acid.

Blood histamine was determined by the Code (1937) modification of the Barsoum and Gaddum method (1935). Tissue histamine was determined by a modification of the method of Best and McHenry (1930). The modification consisted of boiling the tissues in the hydrochloric acid over an open flame for one hour instead of placing them in a boiling water bath. It was found that the tissues became fragmented much more easily and that drying "in vacuo" was hastened. There was no difference in the final histamine content of tissues extracted in this way as compared to that of similar tissues prepared by the original method. All assays were carried out on the isolated guinea-pig ileum preparation suspended in Tyrode solution at 38°C. to which atropine was added in a concentration of 1×10^{-6}. Most of the tissue extracts were incubated with a standard histaminase[2] preparation as further test of the nature of the active substance. All values are expressed as histamine base in gamma per cubic centimeter of blood or gamma per gram of tissue.

Results. The histamine content of the blood, stomach, small intestine, kidney, spleen, liver and lung was determined in the first five animals of both the control group and the adrenalectomized animals maintained on saline. It was noted that no marked change occurred in the histamine content of the blood, kidney or spleen of the adrenalectomized animals as compared to that of the controls. The average histamine content of the blood was found to be 0.03 y/cc., that of kidney 0.42/gram and that of spleen 5.3 y/gram. A marked increase in concentration, however, was noted in the histamine content of the small intestine and stomach, and a moderate increase in that of the liver and lung. Accordingly the histamine content of only stomach and small intestine was determined in the rest of the animals of both groups and that of the liver and lung in a smaller number. The individual determinations for these latter tissues are given in table 1. It will be noted that there is a marked increase in the histamine

[2] Supplied by the courtesy of the Winthrop Chemical Company and Dr. H. Cave, Montreal, Canada.

content of the tissues of the adrenalectomized animals, that of the stomach being 185 per cent and small intestine 208 per cent of the control values. There is also a moderate increase in the liver and lung to 125 and 120 per cent of the control values respectively.

When water is substituted for saline as in the third group of animals, an even greater increase in the histamine content of the tissues occurs.

TABLE 1

Average histamine content of the stomach, small intestine, lung and liver of control rats, adrenalectomized rats maintained on saline and adrenalectomized on saline for seven days and on water for an additional four days

STOMACH			SMALL INTESTINE			LIVER		LUNG	
Controls	Adrenalectomized and maintained on saline for 11 days	Adrenalectomized and maintained on saline for 7 days and on water for 4 additional days	Controls	Adrenalectomized and maintained on saline for 11 days	Adrenalectomized and maintained on saline for 7 days and on water for 4 additional days	Controls	Adrenalectomized and maintained on saline for 11 days	Controls	Adrenalectomized and maintained on saline for 11 days
9.25	26.4		1.09	6.6	10.7	1.5	2.4	7.8	11.48
9.6	17.3		4.4	8.0	11.8	1.7	2.7	7.3	4.8
15.0	19.5		[illegible]	10.0	9.5	1.2	1.85	8.5	11.3
13.3	20.0		3.0	6.15	10.8	1.0	1.72	5.8	9.7
15.0	24.1		2.6	6.15	8.5	1.3	1.62	6.5	9.5
12.6	31.0	44.4	4.4	5.1	9.5	1.3		9.1	5.8
14.8	22.9	44.6	2.6	4.0		4.3		5.5	9.2
20.0	21.0	43.0	3.1	5.0				6.8	12.7
8.0	20.1	44.0	3.6	7.4					12.7
10.1	26.9	42.8	3.2	8.0					
10.6	25.0	45.2	3.6	7.0					
15.6	31.8		3.1	6.5					
Average 12.9	23.9	44.0	3.2	6.6	10.1	1.6	2.04	7.4	9.68
Per cent of normal.....	185	334		208	310		125		120

The stomach contains 334 per cent and the small intestine 310 per cent of the control values (see table 1).

As further evidence in favour of the active substance being histamine and not one of the other vaso-dilator substances the extracts were assayed both by the guinea-pig ileum method and by the blood-pressure method on the atropinized cat under Dial anesthesia. As a general rule the tissue extracts were too concentrated for direct assay on the guinea-pig ileum and they were therefore diluted. Examples of assays using both methods for tissues from normal and adrenalectomized animals are shown in figure

1. As a final criterion that the substance involved was histamine, it was found that when the extracts were incubated with a standard histaminase preparation, their potency was destroyed.

Discussion. The increase in histamine content of the stomach and small intestine could be due theoretically to an increased formation of the substance in these tissues, to an increased transfer of histamine to them from other tissues or from the lumen of the gastro-intestinal canal, or to a lessened rate of destruction or excretion of histamine by these organs.

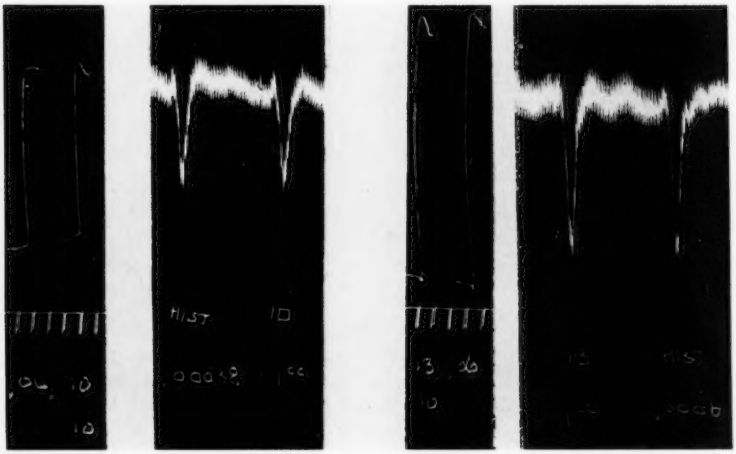

Fig. 1. Assay of extracts of intestine. The assay of extract no. 10, which is of small intestine from a normal rat is shown. At A assayed on the guinea-pig ileum it contains 0.04 y/cc. diluted 1:10. At B assayed by the cat blood pressure 1 cc. original extract contains 0.38 y. In the same figure, the assay of extract no. 13 which is of small intestine from an adrenalectomized rat is shown. Assayed by the guinea-pig method it contains 0.06 y/cc. (shown at C) in a dilution of 1:10. At D it is shown assayed by the cat blood pressure method where 1 cc. original extract contains 0.6 y.

Relatively little is known concerning the mechanism of histamine formation in tissues. Best, Dale, Dudley and Thorpe (1927) were the first to demonstrate its presence in fresh tissue. It is supposedly formed in the gastro-intestinal tract by bacterial action (Koessler, Hanke and Sheppard, 1928) although recent work points to a formation at this site by some other means (Dragstedt, Ramirez de Arellano and Lawton, 1940).

Since adrenalectomy greatly reduces the ability of the tissues of the rat to destroy histamine, it may be that this is responsible for the increase in the histamine content of the tissues observed in the present experiments. The rat stomach does not contain histaminase. It is possible however that

the reduction in histaminase in those tissues which contain it which occurs following adrenalectomy (Karady, Rose and Browne, 1940) is responsible for an increase in the histamine content of the stomach, because more histamine is transferred to it. It could also be due to a decrease in the ability of the stomach either to destroy histamine by some means other than histaminase, or to excrete it into the gastric contents (MacIntosh, 1938).

That ulceration of the gastro-intestinal tract may be produced by the administration of histamine has been demonstrated in many animals (Harde, 1932; Walpole et al., 1940). It may be that similar lesions in animals following adrenalectomy (Selye, 1937a) and in patients with Addison's disease (Maranon, Sara and Arguelles, 1934) may be caused by an increase in the histamine content of the gastro-intestinal tract.

Swingle and his collaborators (1933) suggested that there was a marked similarity between adrenal insufficiency and traumatic shock, and the understanding of the relationship of the adrenal gland to the reaction of the organism to damaging influences such as trauma has been greatly clarified by the work of Selye (1937b). He has suggested that the symptoms of adrenal insufficiency and shock may be due to the liberation of a histamine-like substance from tissues. The results of the experiments described in the present paper tend to support this theory in that there is a marked increase in the histamine content of the viscera in which the ulceration occurs. It has recently been demonstrated (Rose and Browne, 1940), that the blood histamine of patients in various types of shock falls to very low levels during the height of the symptoms and returns to within normal levels as the patient recovers. Since it has been shown that histamine increases in the tissues about an area of inflammation (Rocha e Silva and Bier, 1938), and in the abdominal viscera of the dog following acute burns (Kisima, 1938), it is possible that histamine is transferred from the blood to the gastro-intestinal tract, resulting in a decrease of the blood histamine and an increase in that of the gastro-intestinal tract.

There is thus evidence suggesting that histamine may bear a relationship to the production of the symptoms of adrenal insufficiency and shock and that the metabolism of histamine is influenced by the cortex of the adrenal gland.

CONCLUSIONS

Experiments have been done on the rat to show that there is a marked increase of the histamine content of the gastro-intestinal tract and a lesser increase of the histamine content of the liver and lung following adrenalectomy. The metabolism of histamine and its possible rôle in the production of symptoms in adrenal insufficiency and shock are discussed in relation to these findings.

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THE OXYGEN CONSUMPTION OF SKELETAL MUSCLE FROM ANIMALS DEPRIVED OF VITAMIN E[1]

IRVING FRIEDMAN AND H. A. MATTILL

From the Biochemical Laboratory, State University of Iowa, Iowa City

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It has been abundantly demonstrated that the nutritional dystrophy of voluntary muscle in rats, rabbits and guinea pigs on diets deficient in vitamin E can be prevented and cured by the administration of α-tocopherol (1, 2, 3). The progress of this muscular degeneration is accompanied by a marked decrease in muscle creatine and the appearance of creatine in the urine (4). Other changes in muscle composition and function indicate that the normal course of muscle metabolism is altered in the absence of tocopherol (5, 6). Since this substance is required in very small amounts and is easily oxidized under certain conditions the possible participation of tocopherol in the control of cellular oxidations in muscle cells readily comes to mind.

An increased consumption of oxygen by dystrophic muscle of rabbits was first shown by Victor (7), whose findings were confirmed by Madsen (8) and extended to dystrophic guinea pigs. The total metabolism of such guinea pigs appeared not to be increased (9), whereas that of vitamin E low rats was found to be slightly above that of animals on normal diet (10). It was in rats that the connection of vitamin E with muscle degeneration was first definitely established (11) but no information seems to be available as to altered muscle metabolism when rats are deprived of tocopherol except a note by Drummond (12) to the effect that tissues from E-deficient rats exhibited no diminished oxygen uptake as compared with those of normal animals.

The observations reported in this paper are to the contrary effect: in rats, as in rabbits and guinea pigs, the oxygen consumption of skeletal muscle is above the normal when the animals are maintained on a diet deficient in vitamin E. A few confirmatory measurements were also made on dystrophic rabbit muscle.

EXPERIMENTAL. The control animals were maintained on standard diets,[2] deficient rats were reared from weaning on an E-deficient diet for

[1] From a thesis presented by Irving Friedman to the Faculty of the Graduate College of the State University of Iowa in partial fulfillment of the requirements for the degree of Master of Science.

[2] Purina dog or rabbit chow.

six and thirteen months, and young rabbits were made dystrophic in about three weeks on a synthetic diet which included cellulose, casein, sucrose, starch, salts, cod liver oil, lard and 10 per cent of bakers' yeast.[3]

Oxygen consumption was measured in air in Warburg flasks of the Erlenmeyer type; the temperature of the water-bath was 38°. Thin longitudinal strips of the semi-tendinosus muscle (triplicate samples) were rapidly weighed on a torsion balance (±0.01 gram) and transferred to the Warburg vessels along with phosphate-saline to a final volume of 3 cc., and, in the inner compartment, 0.2 cc. of KOH and filter paper. The vessels were allowed to equilibrate in the water-bath twenty minutes before readings were begun; these were continued for four hours. The tissue was then removed from the flask, quickly rinsed with distilled water, placed in weighing bottles and dried over night at 110°C. for weighing. The results are expressed as Q_{O_2} (cubic millimeters of oxygen per milligram dry weight of tissue per hour). The few creatine determinations were made by the method of Rose, Helmer and Chanutin (13).

The dystrophic rabbit tissue was taken for examination when it became evident that the death of the helpless animals was imminent; they were stunned by a blow on the head. Histological examination was considered unnecessary[4]; macroscopically, the muscles were grayish-white, and appeared to have lost their irritability since they never contracted or twitched as a normal muscle does when it is cut. The data in table 1 show that the oxygen consumption of dystrophic rabbit muscle was about 30 per cent above that of normal rabbit muscle. Qualitatively these results are in agreement with those of Victor (7) and of Madsen (8); quantitatively they are lower both for dystrophic and normal muscles, probably because our measurements were made in air whereas theirs were made in oxygen. This fact may also account for our more rapid decline in oxygen uptake with time; the oxygen uptake was most constant during the second hour.

The rats were injected with sodium pentothal and blood was obtained from the dorsal aorta for use in other studies. The six months old animals were such as could be used for assay of vitamin E; all appeared to be normal in outward respects and had full control of their hind legs. It was striking, therefore, to find (table 2) that the Q_{O_2} during the second hour averaged 2.87 for the muscles of these animals as compared with an average of 1.99 for normal muscle, an increase of more than 40 per cent. The difference in oxygen consumption continued during the remaining two hours. Too few creatine determinations were made to support

[3] Courtesy of Northwestern Yeast Co.

[4] The Department of Pathology, Doctors Brinkhous and Warner, have been very helpful in preparing and interpreting sections of muscle from many similar animals.

any conclusions; the differences here were not as great as in oxygen consumption.

Other older (thirteen months) vitamin E-deficient animals appeared somewhat like those described by Ringsted (14) and more recently by MacKenzie, MacKenzie and McCollum (15). The hind legs were spread out and the posterior part of the abdomen dragged along the ground. The gait was erratic and lacked control. The oxygen consumption (table 2) was only slightly above normal and markedly lower than that of the muscles of the younger deficient animals. Judged by the outstanding degenerative lesions in the dystrophy of older rats (16) the active protoplasmic

TABLE 1

Oxygen consumption and creatine content of normal and dystrophic skeletal muscle of the rabbit

CONDITION	WEIGHT	TISSUE CREATINE	Q_{O_2}* OF MUSCLE			
			1 hour	2 hours	3 hours	4 hours
	grams	*mgm /100 gm.*				
Normal	812		1.61	1.31	1.13	1.02
Normal	810	482	1.76	1.58	1.60	1.35
Normal	850	528	1.71	1.50	1.36	1.30
Average			1.69	1.46	1.33	1.22
Dystrophic	794		2.28	1.97	1.75	1.69
Dystrophic	805		2.29	1.88	1.91	1.96
Dystrophic	808	293	1.98	1.97	1.83	1.83
Average			2.18	1.94	1.83	1.83

* Oxygen consumption in cubic millimeters per milligram of dry weight of tissue per hour.

tissue in such muscles must be greatly reduced; their creatine content was very low.

A few preliminary experiments were performed to determine how quickly α-tocopherol might reduce the oxygen consumption of E-deficient rat muscle to a normal level. Five milligrams of α-tocopherol acetate[5] were fed postabsorptively to five months old female rats whose muscles were examined 24, 72, and 120 hours later. In two of the three series the Q_{O_2} was lower 24 hours after administration of tocopherol than at any other time; in the third series it was lowest after 72 hours. Muscle creatine figures were again too nearly normal to be significant.

DISCUSSION. The increased rate of oxygen consumption by the muscles

[5] Generously supplied by Hoffman LaRoche, Inc.

of rats on E-deficient diets is of the same order as that recently found (measured in oxygen) for the diaphragm of rats on diets deficient in riboflavin and in the other heat-stable components of the vitamin B complex (17); no histological changes could be detected in the diaphragm.

TABLE 2

Oxygen consumption and creatine content of semi-tendinosus muscle

SEX	WEIGHT	MUSCLE CREATINE	Q_{O_2} OF MUSCLE			
			1 hour	2 hours	3 hours	4 hours
		Normal rats				
	grams	*mgm./100 gm.*				
♂	372		1.98	1.89	1.71	1.63
♂	308		2.10	1.94	1.77	1.58
♂	196		2.02	1.85	1.62	1.52
♀	260		2.18	1.84	1.73	1.66
♂	230	401	2.15	2.17	2.05	1.89
♀	298	436	2.71	2.25	1.94	1.91
Average....	277		2.19	1.99	1.80	1.70
		Vitamin E deficient rats (five months old)				
♀	216		3.81	2.97	2.51	2.32
♀	200		3.77	3.17	2.82	2.57
♀	204		2.86	2.51	2.00	2.16
♀	206		3.43	3.17	2.59	2.27
♀	216		2.84	2.43	2.12	1.84
♀	208		2.79	2.38	2.19	2.02
♀	204		2.93	2.57	2.29	2.07
♀	230	393	3.39	2.89	2.43	2.00
♀	220	393	3.27	2.71	2.39	2.19
Average....	212		3.23	2.87	2.42	2.16
		Severely paralyzed vitamin E deficient rats (thirteen months old)				
♀	164	302	2.79	2.24	1.94	1.84
♀		331	2.52	2.17	1.88	1.71
♀	200	321	2.44	2.50	2.14	2.04
♀	220		2.49	2.16	2.04	1.75
♂	260	300	2.36	2.00	1.77	1.61
Average....	211	314	2.52	2.17	2.03	1.79

Similarly, in vitamin E deficient animals, Knowlton and Hines (6) found no gross symptoms of dystrophy and only minor histological changes in the muscles, but the gastrocnemius showed decreased maximal contractile power, decreased creatine and increased chloride concentration. A lack of heat-stable members of the vitamin B complex can not be associated

with the increased oxygen uptake in our experiments since the E-deficient diet contained untreated casein and 8 per cent of yeast.

Verzar (18) concluded that vitamin E exerts its effect directly on muscle since creatine excretion in the urine of dystrophic rats decreased immediately upon feeding large doses (200 mgm.) of α-tocopherol and again increased as soon as the administration of α-tocopherol was stopped. The possible rôle of tocopherol in cellular oxidation has been mentioned by several workers (19); the physiological processes concerned appear to be under some nervous control (20), perhaps of a trophic nature.

The interesting suggestion has been made (21) that the existence in tissues of a series of biocatalysts for oxidation accomplishes gradual degradation of a metabolite such that its total energy is released stepwise rather than completely at a single bound. With this figure in mind one might postulate that in the absence of tocopherol one of the intermediate and delaying steps drops out and that oxidation therefore proceeds the more rapidly; in vitro, under some conditions, α-tocopherol is an antioxidant. The nature of the metabolites concerned in this accelerated muscular oxidation and the possible agencies associated with tocopherol are under investigation.

SUMMARY

1. The oxygen consumption of the semi-tendinosus muscle of six months old rats, reared from weaning on a diet deficient in tocopherol, was 40 per cent above that of normally fed rats.

2. Older (thirteen months) animals that were severely paralyzed through lack of tocopherol showed a much smaller elevation of the oxygen consumption.

3. In confirmation of observations of others, the oxygen consumption of the semi-tendinosus muscle of rabbits, made dystrophic by a diet deficient in tocopherol, was higher than that of normal rabbits.

4. The possible significance of these observations is briefly discussed.

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RENAL RESPONSE TO REPEATED ADMINISTRATION OF POST-PITUITARY EXTRACT

HERBERT SILVETTE

From the Department of Pharmacology of the University of Virginia

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Howell in 1898 first showed that the rise in blood pressure obtained after the injection of post-pituitary extract became less noticeable and finally disappeared on repetition of the dose of the extract. He was also unable to maintain an increased blood pressure by the injection of rapidly successive doses of the extract, as were later workers in the field (see Van Dyke, 1936, 1939). In 1914 Herring reported that a second dose of post-pituitary extract, administered after the renal effect of the first dose had passed off, was again active, although the kidney did not expand again to the same extent. Geiling (1926) also noted the fact that diuresis (in anesthetized animals, presumably) occurred after repeated injection of the extract, even though the effect on blood pressure became small or even gave way to a depressor effect. These occasional experiments were performed, however, at a time when there was still much confusion regarding the primary effect and mechanism of action of the antidiuretic hormone; and the question of renal tolerance to repeated injections of post-pituitary extract has apparently not been fully investigated in controlled experiments on unanesthetized animals. The experiments reported herein have been designed to test the effects of successive doses of post-pituitary extracts on animals under physiological conditions.

METHODS. Male white rats, weighing about 200 grams, were used, and 18 animals comprised each experimental group. Food was withheld for 12 hours before each experiment, though water was freely accessible during this time. The method of urine collection has been previously described (Silvette, 1940).

It had previously been determined that fasted white rats allowed water ad libitum drank on the average 7 cc. per 100 grams of body weight and excreted between 5 and 6 cc. of urine per day (Corey, Silvette and Britton, 1939). In order to approximate the normal free intake of water, the experimental animals were therefore given intraperitoneal injections of 2 cc. per 100 grams of body weight every 8 hours in the first group of experiments, and 0.5 cc. every 2 hours in the second group. The urine volumes were recorded at the end of each 8-hour or 2-hour subdivision.

Besides control animals receiving intraperitoneal injections of distilled water alone, other animals were given injections of water plus U.S.P. XI posterior-pituitary solution[1] and of pitressin. The dosage employed was maximal, being 1 U.S.P. unit of the former and 2 pressor units of the latter (i.e., 0.1 cc. of each extract) per 100 grams of body weight every 8 hours in the first group, and 0.5 U.S.P. unit every 2 hours in the second. In the earlier experiments injections of the extract were made subcutaneously at the same time the intraperitoneal injections of water were made; but it later became apparent that combining the pituitary extract and water into a single intraperitoneal injection gave similiar results to administration by divided routes, and the simplified procedure was then adopted.

Results. The results have been analyzed statistically and are shown in tables 1 and 2 as averages of 18 animals ± the probable error of the average. Comparison of results obtained by the use of the U.S.P. pituitary solution, containing both pressor and oxytocic fractions (table 1, B), with those given by the pressor substance alone (table 1, C), indicated that the essential antidiuretic action shown in these experiments was not modified in any way by the oxytocic substance present in the whole lobe extract. It also appeared that unmodified post-pituitary solution produced as marked an antidiuretic effect, cubic centimeter for cubic centimeter, as Pitressin alone, although the pressor activity of the latter is said to be double that of the U.S.P. pituitary solution (Sollmann, 1937).

It will be noted that the initial dose of either of the pituitary extracts inhibited urine flow (table 1, B and C), compared with the control output (table 1, A), and that this same degree of inhibition was uniformly maintained by successive injections throughout a 48-hour metabolic period. That there was no escape from pituitary antidiuresis during this time was shown by the fact that when the extract injections were stopped after the first 24 hours, the antidiuresis also ceased, and the urine output rose to the control level and finally above it (table 1, D).

In a second group of animals the above experiments were repeated, injecting pituitary extract and recording urine output every 2 hours instead of every 8 hours (table 2). In 8 of the 18 pituitary-injected animals the injections of extract were continued for 26 hours and then stopped, whereupon the urine output rapidly rose to the control level. It will be noted that the results obtained using either 8-hour or 2-hour injection intervals were practically identical, indicating a continuous antidiuretic effect (table 1, D compared with table 2, B).

Discussion. The results of the experiments described herein, while interesting in themselves, afford an opportunity for a theoretical discussion

[1] Posterior pituitary solution Squibb, kindly furnished by Dr. John F. Anderson of E. R. Squibb and Sons.

of the mechanism of post-pituitary action which should point the way to future experimentation.

TABLE 1

Effect of repeated doses of post-pituitary extract on urine output

SERIES	FLUID INJECTED*	CUMULATIVE URINE OUTPUT IN CUBIC CENTIMETERS PER 100 GRAMS B. W. AT END OF					
		8 hours	16 hours	24 hours	32 hours	40 hours	48 hours
A	Distilled water	1.4 ±0.08	3.6 ±0.13	4.9 ±0.13	6.3 ±0.16	7.7 ±0.18	8.6 ±0.17
B	Water + 0.1 cc. pituitary extract, U.S.P.	0.8 ±0.09	1.9 ±0.12	2.9 ±0.14	4.0 ±0.16	5.1 ±0 20	5.9 ±0.18
C	Water + 0.1 cc. pitressin	0.9 ±0.04	2.1 ±0.08	3.0 ±0.14	4.1 ±0.16	5.4 ±0.17	6.8 ±0.20
D	Water + 0.1 cc. pituitary extract for 3 inj.; then water alone for 3 inj.	1.2 ±0.07	2.1 ±0.11	3.2 ±0.13	6.6 ±0.21	8.7 ±0.22	9.9 ±0.23

* Two cubic centimeters per 100 grams' body weight every 8 hours. For further details, see text.

TABLE 2

Effect of bi-hourly doses of post-pituitary extract on urine volume

SERIES	FLUID INJECTED*	CUMULATIVE URINE OUTPUT IN CUBIC CENTIMETERS PER 100 GRAMS B. W. AT END OF															
		2 hours	4 hours	6 hours	8 hours	10 hours	12 hours	14 hours	16 hours	18 hours	20 hours	22 hours	24 hours	26 hours	28 hours	30 hours	32 hours
A	Distilled water	0.5 ±0.10	0.9 ±0.07	1.2 ±0.09	1.8 ±0.10	2.1 ±0.14	2.6 ±0.14	2.9 ±0.16	3.6 ±0.13				4.9 ±0.13				6.3 ±0.16
B	Water + 0.05 cc. pituitary extract, U.S.P.	0.3 ±0.03	0.7 ±0.05	1.0 ±0.05	1.1 ±0.04	1.3 ±0.06	1.4 ±0.06	1.6 ±0.07	1.7 ±0.07	2.0	2.3	2.5	2.8	3.0†	3.6	4.4	6.0

* Five-tenths cubic centimeter per 100 grams' body weight every 2 hours. For further details, see text.
† Injection of pituitary extract stopped and distilled water continued alone.

The effect of repeated injections of post-pituitary solution in steadily inhibiting urine flow is in marked contrast to the inability of the extract to maintain an increased blood pressure even on practically continuous injection. It is generally assumed that both pressor and antidiuretic actions

of posterior lobe extracts are due to a single hormone (Van Dyke, 1939), which has a stimulating action on both "pressor" and "antidiuretic" receptor mechanisms, causing the smooth musculature of certain blood vessels to contract, and the tubule cells of the kidney to reabsorb water at a higher rate. It seems probable that the hormone produces its typical effect only as long as it remains in contact, in sufficient concentration, with the receptor mechanisms. Thus, after subcutaneous or intramuscular injection of the extract, there is generally no pressor response (Van Dyke, 1936), apparently because absorption is so slow that at no time are the blood-vessel walls bathed in a sufficiently high concentration of the hormone to cause contraction of the musculature. The tubule cells seem to be affected by a much lower concentration, for subcutaneous injection in the present experiments produced typical antidiuretic effects, while presumably no simultaneous increase in the blood pressure took place.

The question of tolerance would seem to be bound up with the difference in sensitivity. The smooth musculature of the blood vessels appears to be sensitive only to relatively high concentrations of the hormone, and regains its sensitivity very slowly after once being acted on by an effective concentration. The renal tubule cells, on the other hand, would seem to retain their sensitivity indefinitely unimpaired. That the tolerance acquired by the blood vessels should not be shared by the tubule cells is physiologically necessary according to the theory that water balance is maintained by the antagonism between an antidiuretic pituitary hormone and a diuretic cortico-adrenal hormone, the one operating to increase and the other to decrease tubular reabsorption of water (Silvette and Britton, 1938). In order for such an antagonism to be effective, the tubule cells would have to be constantly sensitive to changing amounts of the two hormones.

SUMMARY

The inhibitory effect on urine flow of the antidiuretic hormone of the post-pituitary is maintained, on repeated injections either 2 or 8 hours apart, for at least 48 hours, and during this time no tolerance for the extract is developed. When pituitary extract administration is stopped, the antidiuresis promptly subsides and the urine output reaches the control level. The physiological significance of these observations is discussed.

Post-pituitary solution (U.S.P. XI) and Pitressin have similar effects on urine output, indicating that the oxytocic fraction present in the U.S.P. extract is without influence on the results.

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THE MECHANICS OF GASTRIC EVACUATION[1]

J. M. WERLE, D. A. BRODY, E. W. LIGON, JR.,[2] M. R. READ AND J. P. QUIGLEY

From the Department of Physiology, Western Reserve University Medical School, Cleveland, O.

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According to the laws of hydraulics, material in the gut tends to move from a region of higher intralumen pressure to one of lower pressure. The rate of translocation of material from one cavity to another may be expressed as a function of this differential pressure and the resistance between the two regions: Rate of flow $= K \frac{\text{Pressure A} - \text{Pressure B}}{\text{Resistance to flow}}$. Accordingly, the relation of pressures in the pyloric antrum and duodenal bulb plays an important rôle in the process of gastric evacuation.

Although antral pressure in excess of bulbar pressure is essential for gastric evacuation, expulsion of gastric contents will not necessarily obtain whenever this gradient develops. The pressure, P, developed within a cavity is related to the time-rate of volume change in the cavity, dv/dt, and, R, the resistance to the escape of contents, as shown by the formula $P = (dv/dt)R$. Thus, an increase in resistance interferes with propulsion but favors the development of pressure.

Several of the factors involved in gastric evacuation were investigated as follows: The pressures in the antrum and bulb were accurately measured by the method of Brody, Werle, Meschan and Quigley (1). We employed trained dogs provided with permanent metal cannulae giving access to the stomach and duodenum. Through these cannulae we introduced rubber tubes carrying open plastic recording tips. The open ends of these were arranged to lie 3 to 4 mm. at either side of the pyloric sphincter (fig. 1). The tips contained lead foil inserts to permit roentgenological localization. Pressures from the antrum and bulb were recorded on the photokymograph by optical capsules. Barium sulfate incorporated with mush was fed to the animals and simultaneously with the pressure studies, fluoroscopic determinations were made of the time of 1, antral peristaltic waves from their origin near the incisura angularis to their termination at the sphincter (multiple waves, when present, were recorded separately, fig. 2, B);

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[2] Department of Pharmacology, The George Washington University, School of Medicine.

2, passage of material through the sphincter; 3, bulbar filling, and 4, bulbar evacuation. Several observers were inclosed in a light proof cabinet to observe the fluorescent screen and each observer recorded the phenomenon assigned by pressing a key. A mirror was thus exposed which projected a beam of light to the photokymograph where antral and bulbar pressures were being recorded. The time of shortening of the sphincter diameter from the beginning of contraction to the beginning of relaxation was like-

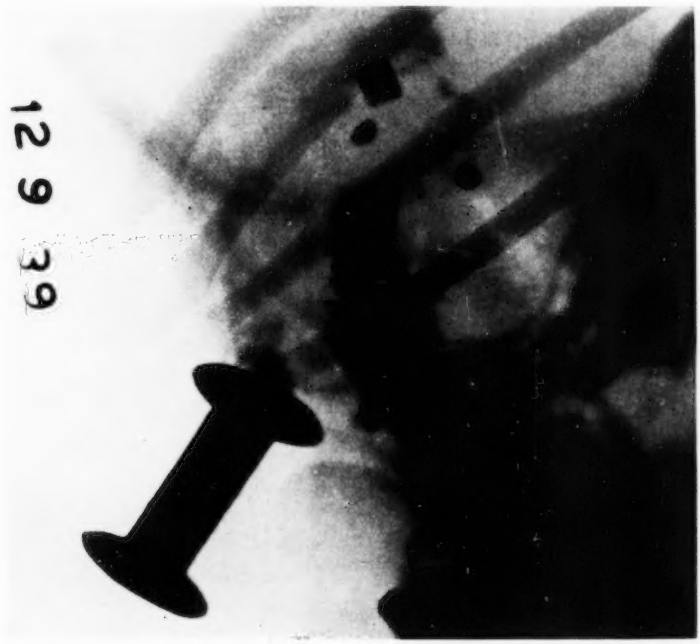

Fig. 1. Reproduction of a radiograph taken from a dog immediately after a barium meal was fed. The small oval shadows indicate the position of shot attached to the serosa at either side of the pyloric sphincter. The cylindrical object below the shot marks the antral recording tip; the bulbar tip is above.

wise recorded by observing the fluoroscopic shadow of lead shot attached to the serosa at either side of the sphincter (fig. 1). The personal factors in observation were minimized by frequent rotation of the experimenter's duties. Registration of the time of pressure changes is entirely accurate but the time relations of the fluoroscopic observations is less satisfactory. A lag in the registration time of beginning and termination of these events is due partly to the reaction time of the recorder (ca 0.20 sec.), but more significant, especially in the registration of shot movements, is the fact

that certain changes in activity of small magnitude are not promptly appreciated.

Indirect information regarding pyloric sphincter activity can be derived from an examination of antral and bulbar pressure. The sphincter probably is firmly closed and resistance to flow is great when antral and bulbar pressures differ markedly, but when the pressures approach each other, communication between the two cavities may or may not be present.

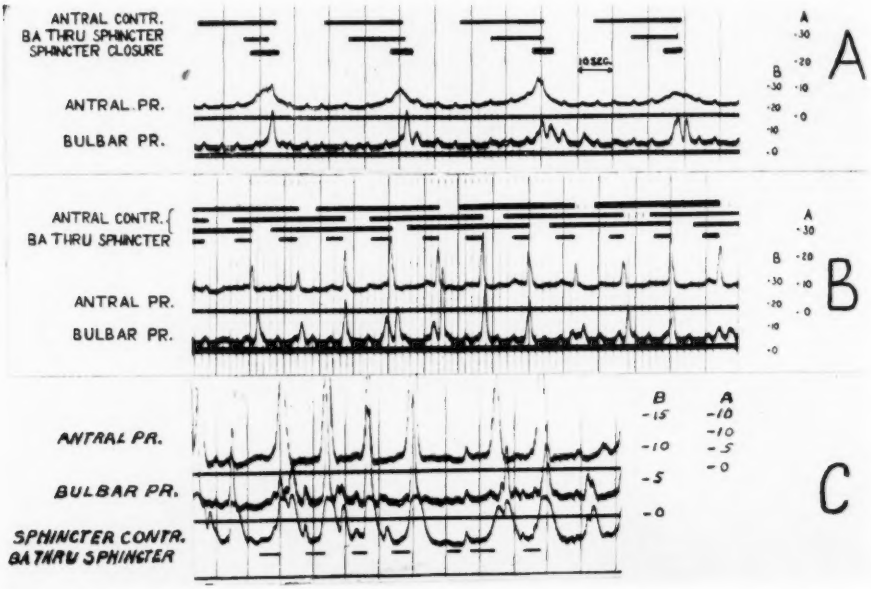

Fig. 2. Interrelation of the events occurring during the gastric evacuation of a meal of corn meal mush and $BaSO_4$. A. When single peristaltic waves pass over the antrum, recorded by a single set of signal bars. Study of sphincter behavior by observing shot movements. B. When three antral waves pass progressively over the antrum, indicated by three sets of signal bars. C. Study of sphincter behavior by optical registration from a sphincter balloon.

When the sphincter is relaxed, the pressures in the two regions should be similar though not necessarily identical, for sufficient resistance in the sphincter region may be anticipated from the anatomical conformation, etc., to explain moderate differences in the pressures in the antrum and bulb.

Measurements of the external diameter at the sphincter are obtained from observations on the shot position, but information regarding the internal diameter of the sphincter would be preferable. The two distances

will be related, but since the sphincter muscle thickens during contraction, the degree of sphincter closure will be greater than is indicated by the decrease in the external diameter. It would be advantageous to have observations of the tone and contractions of the sphincter musculature. The sphincter diameter is the sum of this motor activity plus those factors tending to produce passive sphincter distention. Usually in our records these forces can be differentiated. For example, in figure 3, IV, there are no significant forces tending to produce passive distention, therefore the widely separated shot indicate sphincter relaxation. In this report we

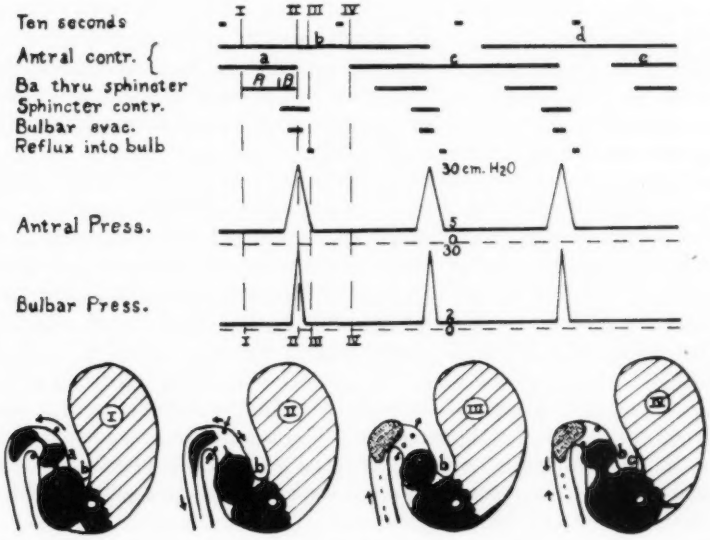

Fig. 3. Evacuation-pressure cycle schematized in four phases. The sketches depict the events at each phase of the cycle at the time indicated by the vertical line designated by the corresponding Roman numeral.

have used this method when feasible to state the type of sphincter activity. We are positive the sphincter is patent only when it is both relaxed and passively dilated, as in figure 3, I, and therefore have applied the term "open" only when this condition obtains. We obtained additional information regarding sphincter activity from a 5 x 7 mm. crescent-shaped balloon anchored in the sphincter lumen which registered by means of an optical manometer. Apparently the balloon was unaffected by pressure variations in the antrum and bulb for the records obtained from these regions differed in time, character and magnitude of change. The time of beginning and cessation of sphincter contraction and also the type of

change in sphincter activity were recorded rather accurately, but exact pressure values were not obtained by this method.

After feeding 150 to 1000 grams of strained mush (cooked corn meal and meat), mixed with 45 to 90 grams $BaSO_4$, records were obtained (fig. 2, A, B, C) which characteristically showed cyclic pressure changes in which maxima of about the same magnitude occurred simultaneously in both the antrum and bulb. During each phasic wave the antral pressure was temporarily in excess, for the pressure rise began earlier in the antrum and terminated later than in the bulb. Also, the basal pressures occurring between phasic changes showed a gradient, for antral pressures were 5 to 8 cm. of water and bulbar pressures 2 to 4 cm. Thus, during each cycle, a pressure gradient which could produce gastric evacuation was present during period A, the interval in which pressures in both antrum and bulb were at the basal level; period B, the first portion of the antral phasic wave; period C, the last portion of the antral phasic wave. Evacuation occurred only during periods A and B but not during C.

Although activity in the pyloric region did not follow a rigid behavior pattern, the following sequence of events occurred most frequently. It was observed fluoroscopically that as a peristaltic wave traversed the antrum it propelled gastric contents towards the sphincter. While a single wave involved the proximal antrum there was no escape of material through the sphincter and the pressure in the prepyloric region remained at the basal level. When the wave reached the distal half of the antrum, evacuation began (evacuation period A). The shot were widely separated, a stream of material passed through the sphincter and frequently some returned to the body of the stomach. The head of pressure supplied by the antrum, although it remained at basal level, was evidently adequate for this phase of the evacuation since the resistance to flow through the sphincter and into the bulb was low.

When the peristaltic wave reached the lower antrum, approximation of the shot began, thus indicating contraction of the sphincter. Observations of the rate of bulbar filling failed to demonstrate that this necessarily decreased the evacuation rate. As the sphincter gradually contracted it provided the increased resistance (R in the formula $P = dv/dtR$) necessary for the antral phasic wave. The peristaltic wave progressively cut deeper and moved to regions of smaller diameter, thus increasing R and dv/dt. Therefore antral pressure increased sufficiently to continue the expulsion of contents (evacuation period B) in spite of the contracting sphincter. Bulbar pressure remained at the basal level. As the antral pressure reached higher levels and the sphincter closed completely the very small amount of material remaining in the distal antrum was forced proximally. The antral pressure fell after the peak of the phasic wave

as the distal antrum began to relax. A decrease in resistance distally was not involved for the sphincter usually had not started to relax when antral pressure began to return to the basal level.

Gastric evacuation ceased because 1, the sphincter was closed; 2, the elevated bulbar pressure provided additional resistance; 3, the prepyloric region was essentially empty. A sphincter contraction always accompanied an antral or bulbar phasic wave regardless of whether, as usually happened, the antral and bulbar waves occurred together, or were independent of each other. Termination of antral evacuation usually coincided with the beginning of the plateau of the sphincter wave and also approximately with the middle of the ascending limb of the bulbar wave. Since the several factors already mentioned occur simultaneously it is impossible to state positively that a sphincter contraction acting alone will normally stop evacuation.

Basal pressure persisted in the bulb as it filled during evacuation period A and the first part of period B, then, with the onset of bulbar contraction, the pressure rose sharply to produce the bulbar phasic wave. Since the sphincter at this time was closing and antral pressure was high, regurgitation into the stomach was prevented. Cole (2), as well as Meschan and Quigley (3), obtained evidence that the sphincter serves the important function of interfering with bulbar regurgitation. Apparently, considerable resistance was encountered to movement down the duodenum (e. g., the material passed distally in a narrow stream). This resistance made it possible for bulbar pressure to rise, i.e., in the formula $P = dv/dtR$, R was great. Also, the rapid bulbar contraction was significant, since it augmented the factor dv/dt. The pressure rose sharply to reach a maximum approximately coincidentally with the peak of antral pressure. The notched bulbar pressure waves sometimes observed usually resulted from repeated bulbar contractions for associated expulsion of bulbar contents, as well as the contractions themselves, were visualized. Periodic changes in the resistance to the escape of duodenal contents could give a similar pressure change. Bulbar pressure fell sharply as most of the bulbar contents was expelled and the bulb relaxed. Relaxation of the sphincter was not essential for this fall, for spreading of the shot usually began after the bulbar pressure had returned to the basal level.

During the later half of the interval required to evacuate a meal, a portion of dilute material frequently returned from the second part of the duodenum to the bulb immediately after the bulbar relaxation (fig. 3, III, IV). This phenomenon is similar to the to-and-fro movement of material in the vertical duodenum—"dancing particles" which have been noted by Barclay (4), and to the regurgitation of barium in the same region reported by Todd (5). Apparently the regurgitation noted by us was

associated with considerable relaxation of the bulb for the bulbar shadow at this time was larger though fainter than during the antral evacuation period.

Several types of deviation from the typical pattern of evacuation described above were noted. When a peristaltic wave swept over the antrum, any fraction of the material initially propelled by the wave might return to the body of the stomach. Usually, most of the material was evacuated, but occasionally, as noted by Cannon (6, p. 96), peristaltic waves passed over the antrum for some time without producing evacuation. If the antral waves were infrequent, shallow, and tended to die out before reaching the sphincter, this failure to evacuate was readily explained. When a vigorous peristaltic wave died out near the sphincter, evacuation period A frequently was present without period B or the corresponding antral phasic wave. Period B might also be absent if the sphincter and bulb failed to develop resistance to the expulsion of antral contents. There were also occasions when an apparently vigorous peristaltic wave swept over the entire antrum, a typical antral phasic wave was recorded, the sphincter was relaxed, a pressure gradient from antrum to bulb was present but evacuation was absent. Usually the food was propelled $\frac{2}{3}$ the length of the antrum by a vigorous wave. The food then returned to the body of the stomach while the wave continued and produced a typical phasic wave. Evidently a further factor which has not been studied in this investigation is essential for evacuation.

Study of the shot movement and the sphincter balloon records showed the contraction wave of the sphincter involved 15 to 50 per cent of each evacuation cycle. Fluoroscopic observations indicated that the sphincter effectively stopped evacuation during only $\frac{1}{2}$ to $\frac{1}{3}$ of its contraction period. Evacuation failed to occur throughout the entire interval during which the sphincter was relaxed, e.g., at figure 3, IV, but it was also noted that at this time the prepyloric region was empty. Gastric evacuation occurred only while the peristaltic wave involved the terminal antrum. When a second or third antral wave started before the first died out, emptying occurred only during the period of maximal overlapping of waves (fig. 2B).

The material discharged from the antrum usually lodged temporarily in the bulb, but when evacuation occurred very rapidly the stream might pass directly down the duodenum before the onset of the typical bulbar contraction. In the latter event, bulbar evacuation began 1 to 2 seconds after the onset of antral emptying and usually persisted for several seconds after its termination.

The peaks of antral and phasic pressure waves usually coincided, but slight variations in which either peak might precede were common. More rarely, an interval of several seconds elapsed between peaks and occasion-

ally an independent antral or bulbar wave developed. A contraction of the sphincter apparently was associated with each of these types of pressure waves. Evacuation usually occurred with each modification of the normal pattern, but in addition to the exceptions already considered, the isolated bulbar wave produced neither gastric evacuation nor bulbar regurgitation. With the onset of evacuation period A the rate of discharge rapidly rose to a plateau which was maintained during this period when the pressure gradient was 3 to 5 cm. of water. Of the total quantity discharged in the cycle, $\frac{1}{2}$ to $\frac{2}{3}$ is expelled during this period. As period A was superseded by period B the gradient gradually rose to approximately 25 cm., but the evacuation rate tended to be maintained, apparently because of the balance between the augmenting antral pressure and the increasing resistance to flow offered by the sphincter. The effective pressure (pressure gradient − resistance) probably remained relatively constant until evacuation terminated abruptly usually with completion of the sphincter closure and the development of the bulbar phasic wave.

At the onset of gastric evacuation the differential pressure relation between antrum and bulb usually remained unchanged, but occasionally it slightly rose or fell. Several factors were integrated with those previously considered to determine the pressure at this moment. During evacuation, material was flowing away from the antral recording tip and toward the bulbar tip. According to the Pitot principle this should lower the recorded pressure gradient. This influence should be slight, for in the process of evacuation the dynamics of flow were feeble. Also, with the onset of emptying, a portion of the energy derived from the contraction which might have been converted into pressure, appeared as energy of flow. Thus the antral pressure showed little tendency to rise. The energy expended by the antrum during the evacuation interval probably was greater than during the preceding portion of the cycle.

We prepared differential pressure records by subtracting bulbar pressures from antral pressures. Such records displayed too much individual variation to be of much significance in explaining the mechanism of evacuation. Since antral basal pressure uniformly exceeded bulbar, the contour of the differential records varied chiefly with the magnitude and time relations of the phasic pressure changes. These time relations normally showed considerable variation and in addition the pressures recorded were greatly influenced by the distance between the recording tips. The most striking changes in a differential pressure record were usually observed during the less important portion of the evacuation cycle. A differential pressure record resembling the three phase cycle of Thomas (7) could occasionally be obtained, but it appeared to be a fortuitous rather than a characteristic result.

SUMMARY

Simultaneous optical registration of intralumen pressures of the pyloric antrum and duodenal bulb of unanesthetized dogs combined with fluoroscopic observations of this region was employed in studying gastric evacuation. In each evacuation cycle the first period of evacuation (period A) was associated with a moderate basal pressure gradient from antrum to bulb. During this interval the pyloric sphincter and duodenal bulb offered very little resistance to expulsion. Since the antral pressure remained low, the energy of the antral contraction occurring at that time probably was chiefly transformed into propulsive force. As the accumulation of material in the duodenum and the contraction of the sphincter increased the resistance distally, the advancing antral peristaltic wave elevated the antral pressure and the antral phasic pressure wave was produced. Gastric evacuation persisted (period B) under the augmented pressure head until terminated by several factors, among which was the completely contracted sphincter. Bulbar contraction occurred at this time and caused bulbar emptying, but regurgitation was prevented by the contracted sphincter.

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RECOVERY OF FATIGUED MUSCLE FOLLOWING INTRAVENOUS INJECTION OF POTASSIUM CHLORIDE[1]

HEBBEL E. HOFF, ALEXANDER W. WINKLER AND PAUL K. SMITH

From the Laboratory of Physiology, the Department of Internal Medicine and the Laboratory of Pharmacology, Yale University School of Medicine

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A temporary increase in the height of contraction of normal skeletal muscle is known to follow the close arterial injection of potassium chloride (2, 3, 4, 11). Baetjer observed this increase in fully curarized muscle (2), and concluded that it was therefore due to a direct effect of potassium on the muscle fibers. Since potassium has some anticurarizing action on neuromuscular transmission (3, 4, 11), this conclusion may not be warranted on the basis of Baetjer's experiments alone. However, the demonstration by Brown and von Euler (3) that the increase may be elicited in previously denervated muscle proves conclusively that it must at least in part be due to a direct effect on muscle, in addition to any facilitation of neuromuscular transmission.

Wilson and Wright (11) failed to elicit any appreciable increase in the height of contraction following the intra-arterial injection of potassium in fatigued muscle. Winkler, Hoff and Smith (13), however, have observed that the height of contraction of skeletal muscle is well maintained during the course of the intravenous injection of potassium chloride; late in the course of the injection the height of contraction may even be greater than at the beginning. This is remarkable since fatigue alone would cause a decrease in the height of contraction during the course of the experiment. It accordingly seems probable that potassium injected intravenously does affect fatigued muscle. The experiments to be described deal with the effects of such injections on fatigued muscle.

METHODS. Cats deeply anesthetized with nembutal were used throughout. In the two experiments with neuromuscular transmission the left quadriceps, after being isolated by appropriate nerve and tendon section, was arranged to pull against a torsion wire myograph writing by means of a straw on a smoked drum. The nerve was stimulated by supramaximal

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break shocks delivered to the motor nerve every two seconds from a thyratron stimulator. The resulting twitches were recorded on a slowly revolving drum. The circulation to the muscle was intact, and the muscle was kept warm and moist by means of a cellophane shield and a constant drip of warm Ringer's solution. After a period of stimulation sufficient to cause marked reduction in the height of the twitches, an isotonic solution of potassium chloride was introduced through a cannula into a leg vein at the rate of 2 to 5 cc. per minute. Injection was not continuous, however, but was interrupted for several minutes between each 5 cc. aliquot

TABLE 1

Potassium chloride solution (0.154 M) injected intravenously into cats

EXPERIMENT	TYPE OF STIMULATION	PERIOD OF FATIGUE	TENSION* DEVELOPED AFTER FATIGUE (PER CENT OF INITIAL VALUE)	TENSION* DEVELOPED AFTER KCl INJECTION (PER CENT OF INITIAL VALUE)	VOLUME OF KCl SOLUTION INJECTED
		min.			*cc.*
1	Neuromuscular	69	63	99	45
2	Neuromuscular	325	75	92	20
3†	Direct muscular	0		138	85
4†	Direct muscular	0		100	25
5	Direct muscular	240	46	64	15
6	Direct muscular	95	75	85	55
7	Direct muscular	28	82	164	25
8	Direct muscular	31	75	127	35
9	Direct muscular	15	68	110	60
10	Direct muscular	95	48	77	60
11‡ (a)	Direct muscular	85	46	91	25
(b)		78	45	93	30
12‡ (a)	Direct muscular	45	49	81	25
(b)		60	61	68	25

* The initial tension developed is taken as 100 in each instance.

† No fatigue; control.

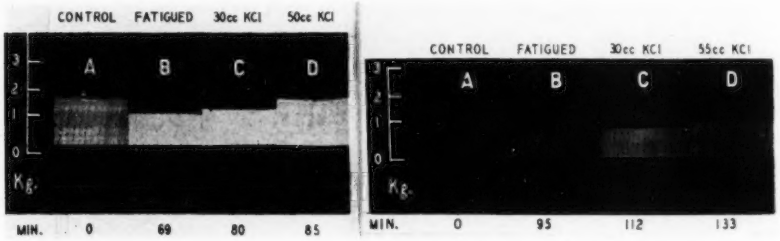
‡ Two periods of fatigue and of injection.

of solution. Injections of 5 cc. amounts were repeated in this manner until death of the animal from the toxic effects of potassium on the heart (12).

In the great majority of the experiments the sciatic nerve had been sectioned 4 to 7 days previously. The combined gastrocnemius-soleus muscle was stimulated directly through several tinned pins inserted into the belly and the tendons of the muscle. Care was taken that supramaximal break shocks only were employed throughout the course of the experiment. Otherwise the technique was identical with that employed in the experiments in which nerve stimulation was used.

RESULTS. Results were uniform in both the neuromuscular and in the direct muscle stimulation series, and are summarized in table 1. Each

intravenous injection of 5 cc. of isotonic potassium chloride was followed within a few seconds by a progressive increase in the height of the twitches, which lasted as long as injection continued and for a few seconds thereafter. The height of contractions then declined somewhat, but did not fall back to the previous level. Each successive injection thus resulted in an additional gain, until in some experiments the original height of contraction was regained and even surpassed, despite several hours of muscular twitching. In other experiments the heart stopped before the height of contraction regained its initial level. Figure 1A is taken from an experiment with neuromuscular stimulation, figure 1B from an experiment with direct muscle stimulation of a previous denervated muscle. Segments of record taken initally, after fatigue, and again after different amounts of potassium are placed side by side for purposes of comparison. The rapid recovery of



A B

Fig. 1A. Neuromuscular stimulation. Restoration of the height of contraction of fatigued muscle following the intravenous injection of isotonic KCl. (Cat, quadriceps muscle, femoral nerve stimulated.)

Fig. 1B. Muscle (gastrocnemius-soleus) denervated 125 hours previously, stimulated directly by multiple electrodes. The intravenous injection of KCl restores the height of contraction of fatigued muscle as readily as in figure 1A.

height of twitches after potassium, in spite of further contraction and fatigue, is clearly seen in both types of experiment.

Discussion. These experiments together with those described previously (2, 3, 4, 11) indicate that an excess of potassium in the circulating fluids prevents the diminution of tension which normally occurs after a series of twitches, or, if such diminution has already occurred, restores the vigor of contraction to normal. Since it is obtainable as readily in the denervated muscles as in those stimulated through the motor nerve, this effect is evidently due chiefly to the action of potassium on the contracting muscle fibers themselves. These observations may be related to the known liberation of potassium from muscle during muscular activity of the rhythmical type studied here (5, 7, 8), which is proportional to the duration and intensity of the contraction. It has been suggested that this loss of potassium may itself be one of the factors responsible for the progressive

decrease in the intensity of contractions in fatigue (6). If this were so, anything that would favor the continuous replacement of muscle potassium might be expected to prevent muscle fatigue or to restore contractions in a muscle already fatigued. It is therefore a reasonable, though unproven, hypothesis that potassium injected intravenously restores the vigor of contraction of fatigued muscle by replacing the potassium which had been previously lost during the development of fatigue.

The observation that potassium may at times cause liberation of adrenaline (1, 10) suggests the possibility that the effect of potassium in opposing fatigue may be mediated by adrenaline. This suggestion has little to support it, since potassium injected intravenously at a slow rate causes no rise in pulse or blood pressure (9), indicating that very little if any adrenaline is liberated.

SUMMARY

The decrease in twitch tension which normally appears after a series of rhythmic twitches is prevented by the slow intravenous injection of isotonic potassium chloride. After the decrease in twitch tension has already been established the tension may be restored to normal by the same means.

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GASEOUS NITROGEN AND HELIUM ELIMINATION FROM THE BODY DURING REST AND EXERCISE

A. R. BEHNKE[1] AND T. L. WILLMON[1]

From the Laboratory of the Experimental Diving Unit, Navy Yard, Washington, D. C.

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Data are presented showing 1, the helium content of the body following a three and one-half hour exposure in a helium-oxygen atmosphere; 2, the curve of helium elimination when air or oxygen is breathed; 3, the increased rate of nitrogen and of helium elimination during exercise, and 4, the curve for nitrogen elimination when oxygen is breathed for a period of 15 hours.

A gas dissolved in the tissues of the body will diffuse into the pulmonary air spaces by way of the blood stream in proportion to the difference in partial pressure between the concentration of gas in the body and in the lungs. The breathing either of oxygen or of an oxygen-helium mixture, for example, will bring about an almost complete elimination of the dissolved nitrogen in the body. In all of our tests this principle was utilized in making gas measurements.

Eleven men in good physical condition, usually deep sea divers, served as subjects.

The helium elimination curve. The subjects breathed a gas mixture consisting of 73 to 76 per cent helium, 5 to 7 per cent nitrogen and 19 to 20 per cent oxygen for a period of three and one-half hours. During this period nearly complete saturation was attained as shown by the quantity of gas subsequently eliminated from the body following exposures of six hours' duration.

Following this saturation period the residual helium in the lungs was removed by breathing oxygen or air for a period of three minutes (lung rinsing period). The helium eliminated from the body was then measured over half-hour periods by having the subjects rebreathe either oxygen or air in a closed spirometer system of about 10 liters' capacity.

Analyses of gas samples for helium content were made in the Cady apparatus, operating on the physical principle that nitrogen and gases other than helium will be adsorbed on activated charcoal cooled to the tempera-

[1] Lieutenant, U. S. Naval Medical Corps.

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ture of liquid air. By means of a high vacuum applied to the charcoal the helium can be extracted and subsequently measured in a burette. Analyses of large samples (500 to 1000 cc.) permitted the determination of quantities of helium as small as 1.5 cc. eliminated over a period of a half-hour. With the elimination of less than 1.5 cc. in a half-hour period the end-point was considered to be reached.

The principal sources of variation and error in these experiments were: 1, the total volume of the spirometer system included a fixed value of 1600 cc. for the residual volume and dead space in the lungs; 2, the quantity of helium eliminated during the first three minutes was based in part on a measured value obtained during the third minute and on a computation involving cardiac output and helium solubility in blood. For a man

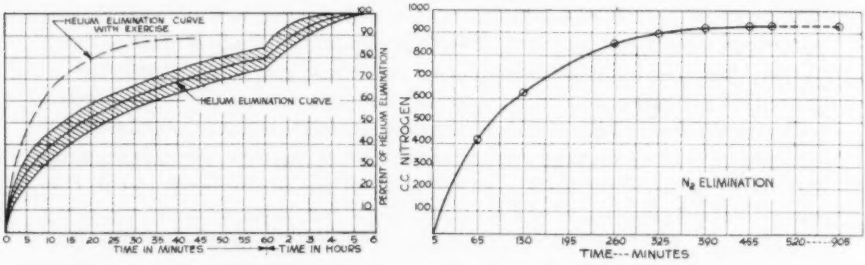

Fig. 1 (left). Percentage of helium elimination from the body plotted against time. Data obtained on 11 deep sea divers who breathed helium-oxygen mixtures for a period of 3.5 hours. Band indicates range of variation of individual results. Helium content of the body is 8.0, 1.3 cc. per kgm., 760 mm. pressure.

Broken line curve illustrates the effect of exercise on helium output.

Fig. 2 (right). Curve showing nitrogen elimination from the body of a diver, age 32, weight 154 pounds, who breathed 99 per cent oxygen in a closed system for a period of 15 hours. The nitrogen eliminated during the first 5 minutes (lung rinsing period) is not included; see table 1.

weighing 150 pounds the value computed for the helium eliminated during the first three minutes (lung rinsing period) was 75 cc. and deviation from this value was considered to be proportional to body weight; 3, an undetermined fraction of the total helium present in the body at the beginning of the saturation period was not measured by our method since it was eliminated through the skin.

The values for total helium content in duplicate tests varied less than ten per cent, and both the values for helium elimination time and helium content of the body per atmosphere of pressure were not altered appreciably by longer saturation exposures at pressures in excess of one atmosphere.

In figure 1 the helium elimination band includes the range of values

around the mean showing the minimum and maximum percentages of helium given off by men at rest in the sitting position.

Since 60 ± 5 per cent of the helium in the body leaves the tissues during the first hour, the time units are expressed in minutes up to 60. The curve is then broken and the time units for subsequent helium removal are expressed in hours.

Considerable variation in desaturation time occurred between subjects, undoubtedly due in part to the difficulty of measuring small amounts of gas collected from the body after 3 hours. Two of the thinnest men weighing 133 and 148 pounds respectively, desaturated in 4 hours in contrast with the desaturation time of 6 hours for two men weighing 202 and 206 pounds respectively.

The helium content of the body. Since it was not feasible to measure the helium tension in alveolar air during the saturation period, we computed the value from the formula,

$$\text{Tension He} = \frac{\text{He content urine}}{\text{He content equil. urine}} \times \text{B-W},$$

where B represents the barometric pressure and W the tension of water vapor.

For it has been shown by Behnke and Yarbrough (1938) that following a change in alveolar nitrogen pressure (and also in alveolar helium pressure) the urinary nitrogen pressure approaches equilibrium with the gas pressure in the lungs after a period of 30 to 60 minutes.

With the tissues of the body in equilibrium with a helium alveolar pressure corrected to 760 mm., the helium capacity of the body based on values obtained from 11 deep sea divers is 3.6 ± 0.6 cc. per pound or 8.0 ± 1.3 cc. per kilogram of body weight.

For comparison, the nitrogen content of a similar group of men was found to be 18 ± 2.0 cc. per kilogram, 760 mm. pressure (Behnke, 1937).

Effect of exercise on helium elimination. The subjects breathed a helium-oxygen mixture while exercising on a stationary bicycle for periods of 15 and 30 minutes respectively. The helium taken up by the body was then measured as in previous tests and compared with the absorption rate of control tests.

Oxygen consumption during exercise was about 3 times the quantity used in control runs, or about 4 times greater than the basal metabolic rate.

The broken line curve of figure 1 represents the values obtained. Exercise over a period of 15 minutes increased helium elimination 60 per cent compared with resting levels, and over a period of 30 minutes, 40 per cent.

In these tests the helium eliminated during the first 3 minutes was not measured, and the values were subject to considerable variation. The

results indicate that the maximum benefit from exercise is derived during the first 15 minutes. A forty-five minute period of exercise, for example, increased helium elimination only 12 per cent compared with control rest periods indicating that exercise of more than 30 minutes' duration does little to increase gas elimination from the body.

The nitrogen elimination curve. The manner of nitrogen removal from the body is shown graphically in figure 2. The data were obtained on a deep sea diver who breathed 99 per cent oxygen for a period of 15 hours while wearing a rubber helmet placed in the circuit of a double spirometer system (figure 3).

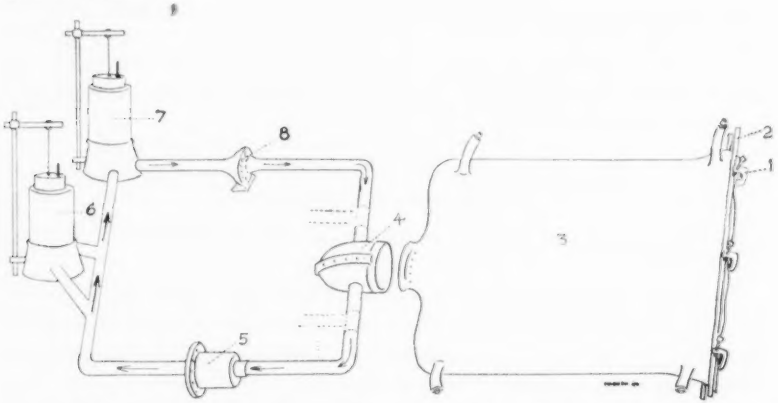

Fig. 3. Diagram of apparatus used to measure gas eliminated from the lungs and gas diffusing through the skin.

1, clamp on iron bars; *2*, used to seal open end of rubber bag; *3*, designed to fit around the body serving as a gas-tight seal; *4*, rubber helmet and collar secured with adhesive tape around the subject's neck; *5*, canister with absorbent for water and CO_2 cooled with ice; *6*, spirometer for measuring added oxygen; 7, spirometer; 8, fan to circulate oxygen, bearing encased in oil to exclude air. Dotted lines indicate connections to a second similar system.

The determination of the quantity of nitrogen eliminated from the body tissues particularly as the end-point is reached, although simple in principle, is a tedious procedure requiring principally an air-tight system for recirculating oxygen and a facepiece that can be worn comfortably for long periods of time.

Since the time limit for wearing a mask pressed tightly against the face is about 6 hours, one of the authors (A. R. B.) devised a close-fitting rubber helmet (dead space 500 cc.) secured with adhesive tape around the neck, which could be worn for a period of 17 hours without discomfort.

The additional spirometer (fig. 3) serves to measure oxygen as well as to facilitate the recirculation of gas.

At the end of each hour following a lung rinsing period of 5 minutes with tank oxygen, a shift was made to an alternate but similar double spirometer system. In this manner it was possible to detect errors incident to faulty apparatus and to maintain percentages of oxygen above 99 in the system after the first 4 hours.

Diffusion of atmospheric nitrogen into the closed circuit amounted to between 20 and 30 cc. per hour in control tests without a subject.

During a test run with a subject breathing in the helmet and surrounded by air, cutaneous absorption of atmospheric nitrogen amounted to between 15 and 25 cc. per hour. This source of nitrogen was obviated by placing the subject in a large rubber bag filled with oxygen.

With reference to the time required for nitrogen elimination, the results are in accord with data obtained by Behnke (1937) in tests conducted at the Harvard School of Public Health. It is observed that the nitrogen is eliminated rapidly during the first 2 hours and then slowly during successive hours. During a period between 9 and 12 hours an end-point within the limits of experimental error (usually $\pm$ 2.5 cc. per hr.) is reached. The results of a single test plotted in figure 1 (first 5 min. of gas elimination excepted) and representative of the data obtained on several men, are listed in table 1.

Nitrogen elimination from the body during rest and exercise periods. Control test. Following a period of three minutes of timed respiration (six deep inhalations per minute) for the purposes of eliminating the residual nitrogen in the lungs, a subject 35 years old, weighing 192 pounds, breathed oxygen through a face mask in a closed spirometer system for a period of 27 minutes daily. There was no preliminary rest period or attempt to regulate the activities of the individual. The results of six consecutive daily tests of nitrogen eliminated between 3 and 30 minutes (subject seated) are the following: 364, 332, 339, 344, 328 and 344 cc. respectively. The oxygen consumption in cubic centimeters per minute for the respective test runs was: 356, 327, 357, 352, 343 and 352.

The results indicate that in 5 out of 6 experiments sufficiently close agreement is attained so as to render the procedure satisfactory. The amount of nitrogen given off by the body during the first 3 minutes of oxygen breathing (lung rinsing period) is indeterminate and presents undoubtedly the greatest source for variation in the values.

Rest and exercise runs. In table 2 are listed the results of nitrogen elimination and of oxygen consumption during rest and exercise for periods of 3 to 13 and 3 to 30 minutes. During the rest period the subject was seated on a stationary bicycle; during the exercise period the bicycle was pedalled at a rate sufficient to increase oxygen consumption two and one half times.

In summary, the results indicate that exercise produces a hundred per

cent increase in nitrogen elimination for the period between 3 and 13 minutes and a 39 per cent increase in the period from 3 to 30 minutes.

Compared with helium test runs, exercise during the first 15 minutes results in a greater percentage elimination of nitrogen. This finding may be explained on the basis of a threefold greater fat-water solubility ratio

TABLE 1

Nitrogen elimination from a deep sea diver, age 32, weight 154 pounds, height 69 inches

(See fig. 2)

TIME	TOTAL ELIM.	N_2 DIFFUSION† INTO SYSTEM	NET N_2
min.	*cc.*		
0–5*	(150)		(150)*
5–65	481	47	434
65–70‡	(25)		(25)
70–130	228	47	181
130–135‡	(12)		(12)
135–195	158	47	111
195–200‡	(7)		(7)
200–260	122	47	75
260–265‡	(5)		(5)
265–325	85	47	38
325–330‡	(2)		(2)
330–390	72	47	25
390–395‡			(−)
395–455	53	47	6
460–520	52	47	5
525–585	47	47	
Body surrounded by oxygen			Total....1076
595–655	46		
660–720	33		
725–785	34		
790–850	24		
Body surrounded by air			
855–905	48		

* Lung rinsing period. Estimated value of nitrogen elimination for this period—150 cc.

† Diffusion of nitrogen into system includes atmospheric nitrogen diffusing through rubber tubing, helmet, spirometer water seal, and nitrogen diffusing through the skin.

‡ Represents a period of lung rinsing whenever a shift was made in spirometer systems. Nitrogen elimination values estimated for these periods.

for nitrogen compared with helium, and consequently the circulating blood has a greater nitrogen reservoir in lipid material to draw upon in comparison with available helium.

Nitrogen elimination when a helium-oxygen mixture is breathed. The addition of helium to the inhaled oxygen did not interfere with the removal

of nitrogen from the tissues of the body. In a typical test a helium-oxygen mixture was breathed for 30 minutes followed by a 90-minute period of oxygen inhalation. The nitrogen given up by the body in the period following helium inhalation was 309 cc. compared with a value of 301 cc. when oxygen was breathed during the initial 30-minute period.

When a helium-oxygen mixture was breathed for a period of 5 hours, 189 cc. of nitrogen were subsequently eliminated compared with 206 cc. eliminated when oxygen was inhaled for the corresponding period of time. Whether or not complete nitrogen removal from the body could be brought about through inhalation of combined helium and oxygen was not tested.

Application of experimental data. The elimination curves for helium and for nitrogen indicate that following saturation a helium dive will require about one-half the period of decompression necessary for an air

TABLE 2

Gaseous nitrogen elimination and oxygen consumption during periods of rest and exercise

SUBJECT	AGE	HEIGHT	WEIGHT	B.M.R.	TOTAL NITROGEN ELIMINATION				OXYGEN CONSUMPTION, CC./MIN.			
					Rest		Exercise		Rest		Exercise	
					3-13′	3-30′	3-13′	3-30′	3-13′	3-30′	3-13′	3-30′
A	35	73	182	276	145	314		417	358	420		1053
B	32	71	175	274		262		361	342	312	614	531
C	31	72	187	283	103	287	194	443	380	403	772	1049
D	29	72	168	270	119	304	268	389	349	346	693	808
E	31	71	190	277	135	271	274	534	354	328	729	649
F	31	69	163	248	157	293	270	395	328	324	973	1088
G	38	71	180	276		364		403	406	383	819	1001
H	30	74	201	297		380		498		355	973	1135

dive. For short exposures, however, we have found that the decompression time is about the same for both the air and the helium dive.

About 75 per cent of the total body nitrogen is eliminated at a comparatively rapid rate and hence does not usually contribute to the formation of "bends." There is however a relatively small amount of gas dissolved in the bone marrow that requires many hours for proper elimination. At a depth of 90 feet, for example, 10.5 hours of air decompression were required following a 9-hour exposure (probable saturation). On the other hand a 2-hour exposure (75 per cent saturation) at the same depth required only 60 minutes for decompression.

In tissues other than the bone marrow and the spinal cord, gaseous diffusion and a greater circulation of blood tend to equalize nitrogen pressure throughout the body (Behnke, Thomson and Shaw, 1935). In the bone marrow and spinal cord the greater nitrogen uptake due to high fat

content, the limitation of diffusion by bony walls, and the sluggish circulation (Campbell and Hill, 1933) appear to be the factors responsible for the slow decompression necessary after long exposures in atmospheres of compressed air. Helium possessing lesser solubility in fat compared with nitrogen should materially hasten decompression if breathed during prolonged exposures in high pressure atmospheres.

In order to augment the removal of absorbed nitrogen during the decompression of deep sea divers, either oxygen or helium-oxygen mixtures can be used. The advantage of the helium-oxygen mixture is that it is not toxic up to depths of 500 feet, in contrast with oxygen which induces toxic symptoms when inhaled at greater depths than 60 feet after prolonged exposures. On the other hand, the absorbed helium in replacing some of the nitrogen will tend to limit the speed of the diver's ascent.

The inhalation of 99 per cent oxygen supplied from commercial tanks for periods as long as 17 hours without eliciting toxic symptoms is considerably in excess of previously reported results. However, the subjects were in a state of complete relaxation, the inhaled oxygen was cooled to between 75° and 80° dry bulb temperature, and the relative humidity was maintained at about 50 per cent. It may be significant also that periods of forced respiration which were associated with substernal distress during oxygen inhalation in 6- to 8-hour tests, were suspended during the 15-hour runs.

SUMMARY

1. At a given pressure the tissues of the body will absorb about 40 per cent as much gaseous helium as nitrogen.

2. The time required for the elimination of the absorbed helium is about 50 per cent of the time required for nitrogen elimination.

3. Exercise hastens gas elimination from tissues but the value of exercise is chiefly during the first 30 minutes.

4. Gas elimination is comparatively rapid from the fluid constituents of tissues; by contrast the bone marrow containing a high percentage of fat requires from 9 to 12 hours for "decompression" after saturation with nitrogen at high pressure atmospheres.

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CUTANEOUS DIFFUSION OF HELIUM IN RELATION TO PERIPHERAL BLOOD FLOW AND THE ABSORPTION OF ATMOSPHERIC NITROGEN THROUGH THE SKIN

A. R. BEHNKE AND T. L. WILLMON

From the Laboratory of the Experimental Diving Unit, Navy Yard, Washington, D. C.

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It was demonstrated by Gerlach (1851) that some elimination of carbon dioxide and adsorption of oxygen was effected through the skin. Subsequent investigations by Schierbeck (1893) and von Willebrand (1902) established the concept of a critical temperature of 33°C. above which carbon dioxide output is proportional to the rise in temperature. Barratt (1897) found that carbon dioxide elimination was doubled at 35°C. compared with the output at 25°C. Shaw, Messer and Weiss (1929) demonstrated a threefold output of carbon dioxide for the same temperature difference. In a subsequent paper Shaw and Messer (1930) reported that there was a critical "effective temperature"[1] of 34° above which the rate of carbon dioxide excretion is accelerated sixfold per unit rise in "effective temperature."

Since carbon dioxide and oxygen are the essential gases in tissue respiration, it was thought worthwhile to measure the cutaneous diffusion of the inert gas, helium, in relation to temperature change and to report for the first time some values for diffusion of atmospheric nitrogen through intact skin.

Essentially we have found that there is also a critical temperature for the increased diffusion of helium through skin, and that the amount of helium absorption is directly related to peripheral blood flow.

METHOD OF PROCEDURE. The unclothed subject lying on a cot was placed in a rubber bag with the head protruding through an air-tight collar. The bag was then inflated with tank helium after an adequate rinsing period of ten minutes.

The helium diffusing through the skin was measured as the amount of gas eliminated from the lungs during rebreathing in the helmet-double

[1] "Effective temperature" as defined by the American Society of Heating and Ventilating Engineers.

Acknowledgment. We desire to express our appreciation to L. B. Lewis, Pharmacist's Mate, First Class, U. S. Navy, for the excellent technical work performed in the helium tests.

spirometer systems described in the preceding paper. Gas samples were taken at half-hour intervals when a shift was made to the alternate helium-free spirometer system. The helium content in the bag around the body varied between 90 and 95 per cent and the helium content in the re-breathed gas was usually less than 1 per cent.

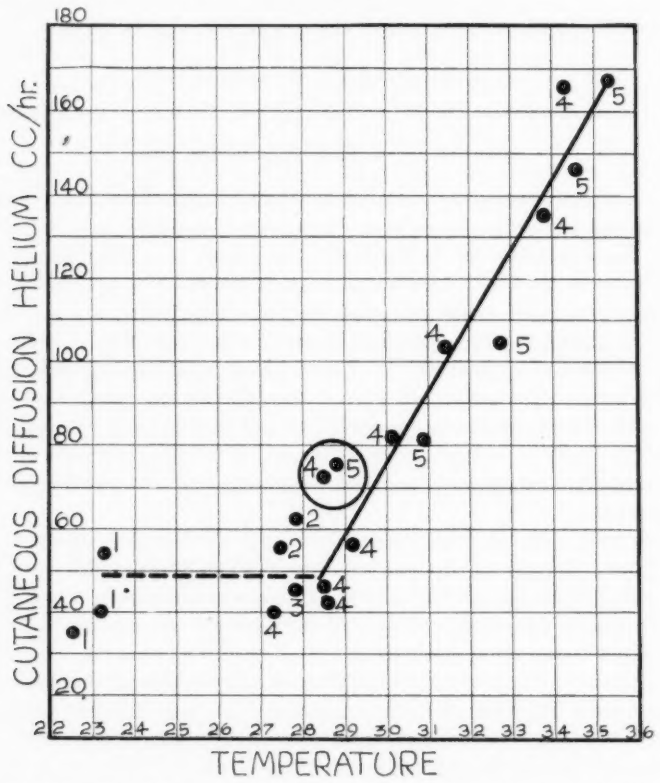

Fig. 1. Cutaneous diffusion of helium in relation to temperature, measured as cubic centimeters of helium recovered from the lungs per hour when the body is immersed in a helium atmosphere, pressure 700 mm. The numbers, 1 to 5, refer to different subjects. The encircled values were obtained after the previously heated ambient helium had been cooled to 29°C.

In the demonstration of nitrogen diffusion through skin it was first necessary to bring about nitrogen desaturation of the body in the manner described in the preceding paper. After the quantity of nitrogen eliminated from the lungs had reached a constant value for a period of several hours, the subject's body enclosed in a rubber bag was surrounded

with air or oxygen. The corresponding fluctuations in the quantities of nitrogen recovered from the lungs relative to the enveloping atmosphere indicated the amount of gaseous diffusion through the integument.

EXPERIMENTAL DATA. When the body was surrounded with helium a period of from 30 to 60 minutes was required to bring about equilibrium between the helium diffusing inward through the skin and the helium eliminated from the lungs.

In a typical test run values for successive half-hour periods following the initial bag rinsing period of 10 minutes were: 9.5, 17.5, 17.1, 20.0, 19.8, 30.7 and 23.7; when the bag helium was replaced by air or oxygen the values for the succeeding half-hour periods in this test were: 15.1, 5.6, 6.0 and 5.0.

TABLE 1

Cutaneous diffusion of helium in relation to temperature, measured as cubic centimeters of helium eliminated from the lungs per hour when the body, enclosed in a rubber bag, is surrounded by helium (700 mm. pressure)

Continuous tests on two subjects

	RUBBER BAG HEATED				BAG COOLED
	Time in minutes from start of helium exposure				
	40–100	100–160	160–220	220–280	365–425
Subject 4, age 31, ht. 65.5 in., weight 140 lb.	S.A. 1.7 sq. m.				
Temperature of bag gas	30.2	31.5	33.9	34.2	28.8
Cubic centimeters He from lungs	82	104	138	167	74
Subject 5					
Temperature of bag gas	31	33	34.5	35.8	29
Cubic centimeters He from lungs	81	105	146	173	75

When the temperature of helium in the bag was 27°C. to 28°C. about 40 to 60 cc. of helium diffused through the skin per hour (fig. 1), helium pressure in the bag being approximately 700 mm.

When the helium in the bag was heated to 35°C. cutaneous diffusion of helium increased to 170 cc. per hour and returned to a value of 70 to 75 cc. per hour when the ambient helium atmosphere was cooled to 29°C. In table 1 the results of continuous tests on two subjects are recorded. The temperature values may be regarded as "effective temperatures" since accumulated moisture in the bag and the profuse sweating indicated a saturated atmosphere.

DISCUSSION. In these tests we are satisfied to show that helium diffuses inward through the skin and that there is a linear increase in the diffusion of helium as measured by the amount of gas eliminated from the lungs,

beginning at 28 to 29°C. and amounting to a two and one-half-fold increase at 36°C. compared with the initial value.

Between 22°C. and 28°C. the diffusion rate follows a plateau but the data are insufficient to establish a quantitative relationship. Apart from an increase in peripheral blood flow, temperature rise augments gaseous diffusion through tissue at the rate of one per cent per degree rise starting with 20°C. as unity (Krogh, 1919); this thermal effect however is within the limits of our experimental error.

We do not consider that a close correlation exists between any particular temperature value in table 1 and the corresponding value for helium absorption since there is a time interval required before equilibrium is established between cutaneous diffusion and the elimination of the absorbed gas from the lungs.

Helium diffusion in relation to peripheral blood flow. The abrupt linear augmentation of helium diffusion in the range of 28°C. and upward is explained chiefly on the basis of increased cutaneous blood flow. Having worked out a simple relationship between heat loss and peripheral blood flow, Hardy and Soderstrom (1938) estimated that for the nude, motionless body, blood flow to the skin increases about threefold between 28°C. and 35°C. Below a temperature of 28°C. blood flow was minimal and constant.

Of prime interest is the close correspondence between the computation of peripheral blood flow on the basis of heat loss from the body and on the basis of helium diffusion through the skin. Under the conditions of their tests, Hardy and Soderstrom found that at 35°C. the blood flow to the skin was about 13 liters per hour per square meter of skin surface. In our tests performed under similar conditions with regard to the resting state, values of the same order as those computed by Hardy and Soderstrom were obtained. If, for example, about 170 cc. of helium are recovered from the lungs per hour (table 1), then about 20 liters of blood would be required to transport this quantity of helium from the periphery to the lungs. This calculation is based upon the following considerations, that under equilibrium conditions 1 liter of blood will hold in solution about 8 cc. of helium at a pressure of 700 mm. (Hawkins and Shilling, 1936). Dividing the hourly value of helium eliminated from the lungs by the solubility value for helium in blood gives a quantity indicative of peripheral blood flow.

The surface area of subject 4 (table 1) according to the DuBois height-weight chart is 1.7 sq. m. Excluding the subject's head and that part of the body in contact with rubber and perhaps not entirely available for gaseous absorption, it is probable that the effective area through which the 20 liters of blood flowed was at least 1.2 sq. m. It would appear that measurements of helium diffusion carefully correlated with skin and ambient gas temperatures should give accurate values for peripheral blood flow.

It may be of interest to record that diffusion of helium outward through the skin when a helium-oxygen mixture is inhaled, is of the same order as inward diffusion of helium. Our tests are too few in number however to form a comparison.

Cutaneous nitrogen diffusion. The demonstration that nitrogen diffuses inward through the skin is made possible only by rendering the tissues of the body relatively nitrogen-free as described in the preceding paper and then by surrounding the body either with air or oxygen. In table 2, column 1, for example, the value of 53 represents the quantity of nitrogen in cubic centimeters accumulating in the closed system for oxygen inhala-

TABLE 2

Diffusion of nitrogen through skin as indicated by the hourly values for nitrogen elimination from the lungs when the body is surrounded by air or oxygen

TIME FROM BEGINNING OF TEST	CC. NITROGEN RECOVERED FROM LUNGS PER HOUR		
	(1) Subject 1*	(2) Subject 2*	(3) Subject 2†
hrs.			
7.5	53		
8.5	52 Body in air		
9.5	47	55	73
11.0	46	49	62 Body in air
12.0	33	47 Body in O_2	49 Body in O_2
13.0	34 Body in O_2	46	
14.0	24	41	68 (2 hrs.)
15.0	48 Body in air	60	37
16.0		67‡ Body in air	
17.0			64 (2 hrs.)

* Subjects breathed 99 per cent oxygen throughout test period.

† Subject breathed helium-oxygen mixture first 4 hours, then oxygen for remainder of test period.

‡ Air around the body heated during last half-hour.

tion during the period between 6.5 and 7.5 hours. This value includes atmospheric nitrogen diffusing into the rubber helmet, tubing, and spirometer water seals, nitrogen diffusing through the skin, and residual nitrogen from the tissues of the body including perhaps a small amount of gas absorbed by the blood stream from the alimentary tract.

Control runs without a subject indicated that between 20 to 30 cc. of nitrogen were absorbed from the atmosphere into the oxygen system per hour. When the body was surrounded by oxygen, nitrogen elimination (column 1) decreased from 47 cc. to a quantity between 24 cc. and 34 cc. only to return to a value of 48 cc. when the body was again surrounded by air.

The data in table 2 indicate, therefore, that between 15 and 25 cc. of atmospheric nitrogen (pressure approximately 600 mm.) diffuse inward per hour through a total skin area of 1.72 sq. m. of which about 0.56 sq. m. of surface was in contact with the rubber bag.

Under similar conditions in comparison with the rate of helium diffusion, nitrogen uptake is somewhat less than 50 per cent. This decreased cutaneous absorption rate for nitrogen prevents the attainment of equilibrium between the nitrogen pressure in cutaneous vessels and in the ambient atmosphere.

SUMMARY

1. Diffusion of helium inward through the skin shows a linear increase with temperature in the range of 28°C. to 35°C.

2. This increase in helium absorption can be correlated with the rise in peripheral blood flow above 28°C.

3. Computation of peripheral blood flow on the basis of helium transport from the periphery to the lungs gives values of the same order as those computed by Hardy and Soderstrom on the basis of heat loss from the body.

4. The diffusion of atmospheric nitrogen inward through the skin has been demonstrated for the first time and is somewhat less than 50 per cent of the amount of helium absorbed under similar conditions.

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NITROGEN ELIMINATION AND OXYGEN ABSORPTION AT HIGH BAROMETRIC PRESSURES

T. L. WILLMON AND A. R. BEHNKE

From the Laboratory of the Experimental Diving Unit, Navy Yard, Washington, D. C.

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Measurements of gaseous nitrogen given up by the tissues of the body when oxygen is inhaled at pressures above one atmosphere have not been previously made with the exception of some tests carried out by Campbell and Hill (1931). The purpose of the experiments reported in this paper was to determine the pressure level at which maximum elimination of nitrogen from the body takes place after previous exposure to a high barometric pressure.

METHOD OF PROCEDURE. Following a rest period of 30 minutes deep sea divers continuing at rest were exposed in a steel chamber to a pressure of 44.5 pounds gauge (4 atms. absolute) equivalent to a sea water depth of 100 feet for periods of 60 and 75 minutes. On the basis of previous measurements it was estimated that under these conditions and excluding the gas absorbed during the first 5 minutes, 50 and 55 per cent saturation of body tissues with excess atmospheric nitrogen was effected. It was assumed that under these conditions the degree of saturation of a given individual was comparatively uniform from day to day.

The rebreathing apparatus (fig. 1) for measuring nitrogen elimination and oxygen consumption under increased barometric pressure consisted of a two-opening rubber bag of 110-liter capacity connected by rubber tubing one inch in diameter to a canister containing a carbon dioxide and moisture absorbent. A spirometer of the Benedict type was incorporated in the system for measuring and recirculating the gas. A face mask and valves completed the circuit.

In a typical experiment 50 liters of oxygen were measured into the system which had been rinsed previously to ensure a concentration of 99 per cent. The subject having been exposed to an excess air pressure of 44.5 pounds per square inch for a period of 75 minutes, breathed oxygen for 3 minutes at the rate of 6 maximum respiration cycles per minute for the purpose of removing lung residual air. The subject was then transferred to the closed-circuit system and the barometric pressure was lowered at the rate of 25 or 50 feet per minute to the level designated for the measurement of nitrogen output.

At the end of the 27-minute period of oxygen inhalation it was found safe to reduce the pressure to one atmosphere. The subject was then transferred to a second closed circuit system for additional measurements of nitrogen output and oxygen consumption.

Periodic withdrawal of gas samples permitted the calculation of eliminated nitrogen. The volume change in a system indicated the quantity of oxygen absorbed, a correction being made for the nitrogen diffusing into the system from the body.

EXPERIMENTAL DATA. In table 1 the data refer to 8 test runs on one subject exposed to an air pressure of 44.5 pounds gauge (100 feet) for 75 minutes. It is observed that during the first 27-minute period of oxygen inhalation, there is a maximum elimination of nitrogen between the 50 and

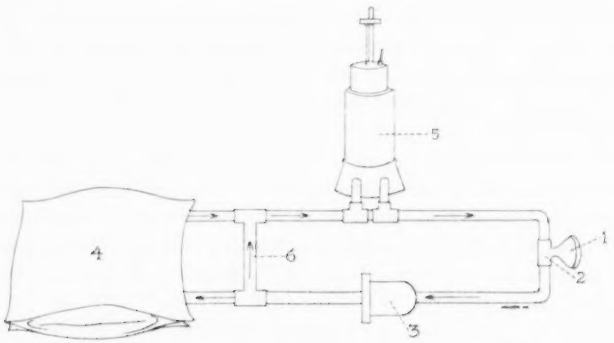

Fig. 1. Diagram of a closed system devised for the inhalation of oxygen to permit measurements of nitrogen elimination at high barometric pressures. *1*, face mask; *2*, valves; *3*, canister containing CO_2 and water absorbent; *4*, 110-liter rubber bag; *5*, spirometer for measurement of gas volume and oxygen consumption.

60-foot levels and a decreased elimination of nitrogen above and below these levels. Of the nitrogen eliminated during the first 27 minutes, approximately six-tenths of the total is given off during the first 10 minutes (3′–13′); of the total amount given off in 84 minutes approximately six-tenths is eliminated during the initial 27-minute period.

Particular significance is attached to the final experiment, table 1. It is observed that nitrogen output at the 100-foot level is 24 per cent lower than at the 60-foot level in a preceding test run; subsequently at the 50-foot level nitrogen output in this test was 73 per cent greater than at the surface level in preceding tests.

In table 2 the values were obtained on subjects who reclined on a mattress in a state of complete rest in contrast with the values obtained on subject C (table 1) who sat in a chair. It is believed that altered posture

TABLE 1

Nitrogen elimination and oxygen consumption of one subject shown at levels from 100 to 30 feet and at surface, following uniform exposure of seventy-five minutes at 100 feet pressure

Subject: C.

DATE	EXPOSURE	STOP	NITROGEN ELIMINATED (CC.)						OXYGEN CONSUMED (CC. PER MIN.)		
			At stop		At surface			Total 3-90′	3-30′	33-60′	60-90′
			3-13′	3-30′	33-60′	60-90′	33-90′				
		ft.									
5/19/39	75 min.: 100 ft.	30	798	1322	728	330	1058	2380	337	262	390
4/24/39	75 min.: 100 ft.	40	831	1335					386		
4/19/39	75 min.: 100 ft.	50	861	1527	544	430	974	2501	377	371	359
5/11/39	75 min.: 100 ft.	55	1023	1534	430	297	727	2261	296	364	361
4/20/39	75 min.: 100 ft.	60	952	1583	560	354	914	2497	369	365	373
5/ 8/39	75 min.: 100 ft.	80	817	1463					405		
5/ 9/39	75 min.: 100 ft.	90	919	1364					455		
5/10/39	75 min.: 100 ft.	100	689	1196	967*	374	1341	2537	558	328	281

* Elimination at 50 ft. level.

TABLE 2

Nitrogen elimination and oxygen consumption of four subjects shown at levels of 100, 50, 20 feet and at surface, following uniform exposure of seventy-five minutes at 100 feet pressure

Mean values given in event of two or more identical tests

SUBJECT	EXPOSURE	STOP	TESTS	NITROGEN ELIMINATED (CC.)			OXYGEN CONSUMED (CC. PER MIN.)	
				At stop 3-30′	Surface 33-90′	Total 3-90′	3-30′	33-90′
		ft.						
S.	75 min.: 100 ft.	20	1	1478	834	2312	232	226
	75 min.: 100 ft.	50	2	1533	957	2590	228	321
	75 min.: 100 ft.	100	2	1415	739	2154	254	260
Z.	75 min.: 100 ft.	20	2	1127	777	1904	214	216
	75 min.: 100 ft.	50	3	1220	809	2029	191	257
	75 min.: 100 ft.	100	1	849	785	1634	361	207
W.	75 min.: 100 ft.	20	1					
	75 min.: 100 ft.	50	1	1587	982	2569	272	300
	75 min.: 100 ft.	100	1	1486	949	2435	316	266
D.	75 min.: 100 ft.	20	1	1081	687	1768	219	264
	75 min.: 100 ft.	50	1	1079	674	1753	208	281
	75 min.: 100 ft.	100	2	1010	754	1764	255	256

may have influenced the results. In addition, pressure was lowered at the rate of 25 feet per minute to the level designated for nitrogen elimination in contrast with a rate of 50 feet per minute (table 1).

Some of the values in table 2 again indicate that a 50-foot level is optimum for nitrogen elimination, while other data show no significant difference between the several levels.

With reference to oxygen consumption the values are fairly constant for depths up to 70 feet. At levels deeper than 70 feet oxygen consumption is apparently increased occasionally as much as 58 per cent, followed by an apparent decrease in oxygen consumption as high as 20 per cent during the succeeding hour at the surface level.

TABLE 3

Nitrogen elimination and oxygen consumption of one subject shown at surface, 44, 50 and 66 feet with subsequent surface measurements, following uniform exposure of thirty minutes at 100 feet pressure

Subject: S.

DATE	EXPOSURE	STOP	NITROGEN ELIMINATED (CC.)			OXYGEN CONSUMED (CC. PER MIN.)	
			At stop 3-30′	Surface 33-90′	Total 3-90′	3-30′	33-90′
		ft.					
3/22/39	30 min.: 100 ft.	0	626	401	1027	274	325
3/24/39	30 min.: 100 ft.	0	1191	499	1690	284	343
3/27/39	30 min.: 100 ft.	0	892	856	1748	269	345
3/28/39	30 min.: 100 ft.	0	1147	511	1658	270	337
3/31/39	30 min.: 100 ft.	44	1343	548	1891	280	309
4/ 3/39	30 min.: 100 ft.	50	1312	565	1877	276	308
3/30/39	30 min.: 100 ft.	66	1341	522	1863	290	327

In a third series of tests one subject was exposed for periods of 30 minutes at a simulated depth of 100 feet. Following a 3-minute lung rinsing period and with the subject breathing in the closed circuit, decompression was effected to the surface or to intermediate levels at the rate of 50 feet per minute.

It should be borne in mind in the interpretation of results (table 3) that periods of exposure of 30 minutes or longer at a depth of 100 feet followed by a 2-minute period of decompression to the surface may give rise to "bends" (air embolism). It is possible that bubble formation in the blood stream might retard nitrogen elimination through a sudden reduction of the pressure head for nitrogen diffusion.

Indicative of bubble formation are the results obtained on March 27 when the values for nitrogen elimination in the first and second periods approached equality in contrast with the results obtained in tests where

stops were made at 44, 50 and 66 feet. In these last three tests decompression was considered to be ample by reason of the oxygen inhalation at high levels, and bubble formation may be considered as highly improbable.

In the tests featured by rapid decompression to the surface bubble formation was manifest by the onset of pruritus and the sudden, excessive fatigue occurring 3 to 5 hours later. These symptoms usually constitute early manifestations of "bends."

DISCUSSION AND APPLICATION OF EXPERIMENTAL DATA. Nitrogen elimination under favorable conditions follows an exponential type of curve with a sharp slope during the first half-hour period. Equal quantities of gas eliminated in the first and second periods bring about a flattening of the curve which suggests either bubble formation replacing a state of supersaturation in the blood stream or a slowing of the circulation induced by oxygen.

The progressive decrease in nitrogen elimination at levels between 60 and 100 feet corresponds closely to the pressure at which oxygen becomes increasingly toxic. One of the striking effects of oxygen at these levels is intense vasoconstriction manifested by facial blanching. It is highly probable that an altered or impaired blood flow consequent upon vasoconstriction is responsible for decreased nitrogen output.

Tolerance to oxygen at high pressures shows considerable variation in different individuals. In the susceptible person typical symptoms are nausea, irritability, and a sense of impending disaster. These symptoms may be followed by violent tonic and clonic muscular spasms comparable to an epileptic seizure (Behnke et al., 1935). At a pressure of 3 atmospheres (66 ft.) narrowing of the visual fields and a decrease in visual acuity are characteristic symptoms.

One of our divers developed an idiosyncrasy for oxygen following repeated exposures at a pressure of two and one-half atmospheres. In contrast to the usual vasoconstriction, an erythema of the face and neck was associated with the inhalation of oxygen even at atmospheric pressure. Subsequently this diver exhibited an allergic type of dermatitis not related to protein sensitivity. Complete remission of symptoms followed the administration of histaminase (Torantil, Winthrop).

It is this variation in susceptibility of different individuals to the toxic effect of oxygen that may account for the different values for nitrogen elimination observed at the 100-foot depth. Another factor for consideration is the well established relationship between carbon dioxide inhalation and oxygen toxicity (Shaw et al., 1934). The effect of oxygen on the elimination rate of nitrogen from individuals completely at rest (table 2) may differ from the effect on an individual in the sitting position possibly on the basis of altered carbon dioxide production.

With regard to the phenomenon of increased oxygen absorption at depths

between 70 and 100 feet (table 1), it is probable that oxygen taken up in solution by the tissues accounts for the increase. In dogs the oxyhemoglobin was not reduced in the venous blood when oxygen was breathed at a depth of 66 feet (Behnke et al., 1934) and Campbell (1929–1930) has demonstrated the great increase in oxygen pressure in the tissues of rabbits at high barometric pressures. The capacity of the tissues to absorb oxygen is about 2.3 volumes per cent for fluids and 11.3 volumes per cent for fat.

These results are of practical application in the decompression of divers. Oxygen inhalation may be started at the 100-foot level and maintained for a period of 30 minutes provided that carbon dioxide is rigidly excluded. Nitrogen elimination however may be retarded at this level because of the toxic effect of oxygen. Safe practice permits prolonged oxygen inhalation at the 50-foot level which under the conditions of our tests is associated with the maximum output of nitrogen from tissues.

During the salvage operations incident to the U. S. S. Squalus disaster, oxygen inhalation at the 50-foot level proved effective in preventing serious injury to divers exposed to high pressure atmospheres (Behnke and Willmon, 1939).

SUMMARY

1. Excess nitrogen gas absorbed by divers exposed in a compression chamber to a simulated depth of 100 feet is usually eliminated more rapidly when oxygen is breathed at a level between 50 and 60 feet in comparison with higher or lower levels.

2. The retardation in the elimination of nitrogen observed at the 100-foot depth when oxygen is breathed is attributed to the vasoconstriction and slowed circulation incident to oxygen inhalation.

3. The increased oxygen absorption accompanying oxygen inhalation at high pressures can be accounted for on the basis of oxygen taken up by the tissues in physical solution.

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THE DEPENDENCE OF THE CARBOHYDRATE, FAT AND PROTEIN APPETITE OF RATS ON THE VARIOUS COMPONENTS OF THE VITAMIN B COMPLEX

CURT P. RICHTER AND CLARENCE D. HAWKES

From the Psychobiological Laboratory, Henry Phipps Psychiatric Clinic, Johns Hopkins Hospital

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Results of previous self-selection experiments have demonstrated the dependence of the carbohydrate, fat and protein appetite of rats on the intake of three of the crystalline components of the vitamin B complex—thiamin chloride, riboflavin and nicotinic acid—and on an extract (Frost and Elvehjem) of W factor (Richter and Barelare, 1939). These experiments have now been repeated without W factor, but with a fourth vitamin B component, B_6, which since then has been crystallized (Stiller, Keresztesy, and Stevens, 1939; Kuhn, Wendt and Westphal, 1939).

It had previously been found that rats grew and thrived on a diet which they themselves selected entirely from an assortment of purified (or nearly purified) substances, offered in separate receptacles (Richter, Holt and Barelare, 1938). This assortment contained at least one representative of each of the substances known to be needed for normal nutrition:

Sucrose	Carbohydrate
Olive oil	Fat
Casein (purified and autoclaved)	Protein
Sodium chloride, 3 per cent	Sodium
Dibasic sodium phosphate, 8 per cent	Phosphorus
Potassium chloride, 1 per cent	Potassium
Calcium lactate, 2.4 per cent	Calcium
Cod liver oil	Vitamins A and D
Dried baker's yeast	Vitamin B complex
Wheat germ oil	Vitamin E

The amount of each one of these substances received by the rats depended entirely on appetite, that is, on individual selections. That the rats actually made beneficial selections we know from the fact that on such self-selection diets they grew and reproduced as well as on our standard McCollum diet, while consuming from 15 per cent to 40 per cent less food as measured in grams. Clearly, they must have eaten only needed

substances. Thus, the rats' voluntary intake or appetite provided a measure of their nutritional needs for the various food substances, minerals and vitamins. With this method studies have already been made on the nutritional needs of pregnancy and lactation (Richter and Barelare, 1938) and of experimental glandular deficiencies, produced by adrenalectomy (Richter, 1937), parathyroidectomy (Richter and Eckert, 1939), and pancreatectomy (Richter and Schmidt, in press).

In the present experiments the rats were kept on the basic self-selection diet, including the 10 substances listed above, except yeast and wheat germ oil—that is, a diet which lacked all components of the vitamin B complex—and were given access to thiamin chloride, riboflavin, nicotinic acid, and B_6 singly and in combinations. In order to obtain information regarding the effects of these different components on the endocrine glands, the rats were killed and autopsied at the end of 40 days on the self-selection diet, that is, when they had reached an average age of approximately 105 days.

METHODS. In these experiments we followed our standard technique used for metabolic and endocrinologic studies. At an average age of 45 days female rats were placed in individual cages, containing a revolving drum with a cyclometer plus a living compartment with a food cup (McCollum diet) and a graduated inverted water bottle. Daily records were taken of activity, food and water intake, and of the vaginal smears. The animals were weighed weekly. After approximately 15 days when base lines had been obtained, the rats were placed on the self-selection diet. This was simply accomplished by removing the small living compartment with the single food cup and water bottle and replacing it with a larger cage which had space for 3 food cups and 8 to 16 bottles for the solutions used in the self-selection diet. After 40 days on these self-selection diets the rats were killed and autopsied. At autopsy all of the endocrine glands were weighed and preserved for histological study.

In these experiments the basic self-selection diet offered the following substances, ad libitum, in separate containers: *solids in food cups*—casein (purified and autoclaved), sucrose; *fluids in bottles*—olive oil, sodium chloride, 3 per cent, dibasic sodium phosphate, 8 per cent, potassium chloride, 1 per cent, calcium lactate, 2.4 per cent, magnesium chloride, 0.5 per cent, cod liver oil, tap water.

One negative control group of 4 rats had access only to this basic diet, while a positive control group of 8 rats had access to dried baker's yeast, in addition to the basic diet, that is, to an assortment of substances from which we had previously found that rats made selections which resulted in normal growth.

The nine experimental groups, usually composed of 4 rats each, had

access to the substances listed above and to thiamin chloride, riboflavin, B_6, and nicotinic acid, singly and in combinations as follows:

1. Thiamin chloride
2. Riboflavin
3. B_6
4. Nicotinic acid
5. Thiamin chloride plus riboflavin
6. Thiamin chloride plus B_6
7. Riboflavin plus B_6
8. Thiamin chloride plus riboflavin plus B_6
9. Thiamin chloride plus riboflavin plus B_6 plus nicotinic acid

Stock solutions[1] of the 4 vitamins (made fresh weekly) were kept in a refrigerator. All food cups and bottles were thoroughly cleaned and refilled twice weekly.

RESULTS. *Preliminary experiments.* The preliminary experiments confirmed our previous experience and showed in addition that rats will take vitamin B_6 and that its ingestion affects protein appetite, growth, and vaginal smears. For these experiments we used 4 rats which for 100 days had been on the self-selection diet, without access to yeast, but with access to thiamin chloride, riboflavin, and nicotinic acid. At this time the rats were underweight, showed dioestrous vaginal smears, and for two months or more had eaten only small amounts of casein and only moderate amounts of sucrose, but large amounts of olive oil. When a 0.02 per cent solution of vitamin B_6 was offered, all the rats took from 1 cc. to 4 cc. per day. The rats gained weight, their vaginal smears again showed cornified cells, however, not with the normal regularity, and they ate larger amounts of protein.

Final experiments. Intake of the 4 vitamins. The vitamins were offered to the rats in the following concentrations: thiamin chloride, 0.02 per cent; riboflavin, 0.0025 per cent; nicotinic acid, 0.1 per cent; B_6, 0.02 per cent. These particular concentrations were chosen empirically on the basis of our own taste.

The rats drank freely of these vitamin solutions. They took considerably more of each than the generally recognized maintenance doses. This may have been due either to the loss of some of the solution in the process of drinking or to a slight deterioration of the solution between changes. Table 1 summarizes the results. At the left it gives the 9 different vitamin choices and in the 4 columns the average daily intake and range of variation of thiamin chloride, riboflavin, B_6, and nicotinic acid in milligrams for the 40-day period.

[1] We wish to express our gratitude to Merck and Company, Inc. for their generous supply of the vitamins used in these experiments.

Carbohydrate, fat and protein appetite. Figure 1 summarizes these results. It gives the average daily carbohydrate (sucrose), fat (olive oil), and protein (casein) intake for the last 20 days on the diets of the two control groups of rats, one offered the basic diet with yeast and one the basic diet without yeast, and also for the nine experimental groups which had access to the basic diet and to thiamin chloride, riboflavin, B_6, and nicotinic acid, singly or in various combinations. The control and experimental

TABLE 1

Average daily intake of vitamins for 40-day period in milligrams

	B_1	RIBOFLAVIN	B_6	NICOTINIC ACID
B_1	0.72 (0.39–1.02)			
Riboflavin		0.11 (0.02–0.20)		
B_6			0.30 (0.05–1.01)	
Nicotinic acid				1.02 (0.91–1.10)
R, B_6		0.08 (0.04–0.11)	0.54 (0.31–0.78)	
B_1, R	0.57 (0.40–0.87)	0.11 (0.09–0.13)		
B_1, B_6	0.30 (0.14–0.53)		0.61 (0.25–1.13)	
B_1, R, B_6	0.65 (0.13–1.67)	0.11 (0.03–0.17)	0.39 (0.33–0.46)	
B_1, R, B_6, N.A.	0.56 (0.24–1.41)	0.17 (0.11–0.26)	0.66 (0.54–0.75)	0.83 (0.49–1.02)

groups are each listed in the order of intake of each of the three foodstuffs. A comparison of the sucrose intake records of the two control groups (solid black rectangles) shows that the rats with yeast ate large amounts (6.6 grams per day), while rats with no yeast ate only minimal amounts (1.3 grams). The riboflavin rats ate only a very small amount of sucrose (0.9 gram), even less than the no-yeast group. The B_6, nicotinic acid, and riboflavin plus B_6 rats showed only slightly larger appetites for sucrose. On B_1 alone or on the combination of B_1 with the other vitamins the sucrose

appetite was always good. On all 4 vitamins the rats ate almost as much sucrose (5.5 grams) as on the full self-selection diet with yeast.

In general, the olive oil or fat appetite varied inversely with the sucrose and casein appetites. The riboflavin rats, which ate the smallest amounts of sucrose (0.8 gram) and casein (0.2 gram), ate the largest amounts of olive oil (2.3 cc.), while the group of rats on all 4 vitamins, which had next to the highest sucrose and casein appetites, had next to the smallest olive oil appetite (0.8 cc.).

The protein appetite of the 2 control groups showed the widest variation of any of the 3 substances. The full self-selection, or yeast, group had an average intake of 3.2 grams, while the no-yeast rats averaged only 0.4 gram. On riboflavin, nicotinic acid, or B_6 alone, or on B_1 and B_6, or on riboflavin and B_6, the protein appetite remained minimal. The protein appetite appears to depend much more on combinations of the vitamins

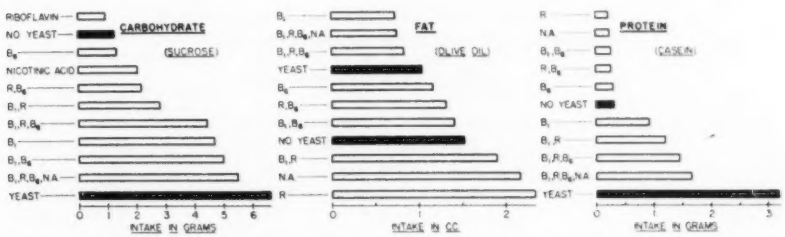

Fig. 1. Graph showing the average daily intake, for the last 20 days on diet, of carbohydrate (sucrose), fat (olive oil), protein (casein) for the 2 control (blacked rectangles) and 9 experimental groups of rats. For each of the three foodstuffs the different groups are listed in order of their average daily intake.

than on any single vitamin, unless possibly it be thiamin. Thiamin and riboflavin constitute the most important double combination, for without these two vitamins the protein appetite remained very low. B_6 added to this combination increased the protein appetite still more, and the addition of nicotinic acid increased it still further, but the combination of all 4 vitamins failed to increase the protein appetite to the average for the rats on the full self-selection diet. Clearly, the protein appetite depends on still other substances present in yeast and not offered in these experiments, such as, for instance, choline, nucleic acid or pantothenic acid.

Table 2 brings out another aspect of these results. It gives the average daily caloric intake of carbohydrate, fat and protein for the last 20 days on the self-selection diet and the average daily total calories. This table lists the various vitamin combinations in order of the average daily caloric intake. It also gives the caloric percentages of the three foodstuffs. The total caloric intake ranged from 17.0 calories for the B_6 group to 36.5

calories for the rats on all 4 vitamins, and 48.5 calories for the control or yeast group of rats. The caloric intake remained low for all the single components as well as for a double component (riboflavin plus B_6) which did not include thiamin chloride.

The second part of the table shows that carbohydrate percentages ranged from 14.3 for the riboflavin rats to 65.3 for the thiamin chloride rats, while the fat percentages varied from 18.2 for the rats on all 4 components to 82.4 for the rats on riboflavin, and the protein percentages ranged from 3.2, 3.3, 3.6 for rats on B_1 plus B_6, riboflavin, and nicotinic acid respectively to 25.8 for the control group of rats on yeast. The percentages of the rats on all 4 components most closely approximated those of the yeast group. The protein percentages showed the greatest discrepancy, 19.0 as compared to 25.8.

TABLE 2

Average daily caloric intake for last 20 days on diet

	CARBOHYDRATE	FAT	PROTEIN	TOTAL	CALORIC PERCENTAGES		
					Carbohydrate	Fat	Protein
B_6	5.8	10.0	1.2	17.0	34.1	59.1	6.8
No yeast	4.8	13.0	1.5	19.3	24.9	67.6	7.5
R, B_6	8.8	11.2	1.1	21.1	41.8	53.0	5.2
Riboflavin	3.5	19.8	0.8	24.1	14.3	82.4	3.3
Nicotinic acid	8.6	18.5	1.0	28.1	30.6	65.8	3.6
B_1	19.2	6.3	3.9	29.4	65.3	21.6	13.1
B_1, R, B_6	18.2	7.2	5.9	31.3	58.2	23.0	18.8
B_1, R	11.5	16.1	4.9	32.5	35.4	49.6	15.0
B_1, B_6	20.7	12.0	1.1	33.8	61.2	35.6	3.2
B_1, R, B_6, N.A.	22.9	6.7	6.9	36.5	62.8	18.2	19.0
Yeast	27.1	8.9	12.5	48.5	55.8	18.4	25.8

Mineral appetite. Table 3 gives the intake of mineral solutions. The intakes of calcium lactate, dibasic sodium phosphate and magnesium chloride of the rats on the various vitamin diets are of particular interest. Their intakes were low in the no-yeast control group and high in the full self-selection or yeast control group. Single vitamins did not have much effect on the appetite for these minerals, but the combination definitely increased it. The group on all 4 vitamins had the highest calcium lactate intake of any of the combinations. The other minerals—sodium chloride and potassium chloride—did not show any consistent changes. Since the rats on 3 or all 4 vitamins showed greatest growth of any of the experimental groups, the increased calcium and phosphorus appetite may be understood as responses to greater needs for these minerals used in skeletal growth.

Effects produced on body weight. Figure 2 summarizes the results produced by the various vitamins on growth. It gives the weights at the end of the 40 days as per cent of the weight at the start. On the full diet (yeast) this averaged 135 per cent; on the no-yeast diet, 62 per cent. On nicotinic acid, on riboflavin, on riboflavin plus B_6, and on B_6 alone the

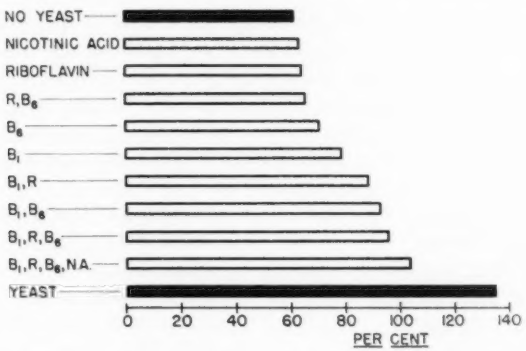

Fig. 2. Graph showing the effects produced by the various diets on body weight. It gives for the 2 control (blacked rectangles) and 9 experimental groups of rats the average body weight at the end of the 40-day period expressed as per cent of the average weight present at the start.

TABLE 3

Average daily intake of minerals for last 20 days in cubic centimeters and grams

	CALCIUM LACTATE		SODIUM CHLORIDE		SODIUM PHOSPHATE		MAGNESIUM CHLORIDE		POTASSIUM CHLORIDE	
	cc.	*gram*	*cc.*	*gram*	*cc.*	*gram*	*cc.*	*gram*	*cc.*	*gram*
No yeast	2.6	0.06	2.0	0.06	1.4	0.11	1.5	0.008	3.2	0.03
B_1	1.6	0.04	3.1	0.09	1.4	0.11	1.7	0.009	3.2	0.03
Riboflavin	2.0	0.05	3.0	0.09	1.5	0.12	2.7	0.014	5.6	0.06
B_6	3.0	0.07	1.4	0.04	1.1	0.09	2.2	0.011	2.5	0.03
Nicotinic acid	3.9	0.09	2.2	0.07	1.0	0.08	1.6	0.008	2.2	0.02
R, B_6	2.0	0.05	2.2	0.07	2.4	0.19	1.8	0.009	3.3	0.03
B_1, R	3.1	0.07	3.9	0.12	2.3	0.18	1.1	0.006	4.7	0.05
B_1, B_6	3.6	0.09	2.5	0.07	2.3	0.18	1.7	0.009	6.5	0.07
B_1, R, B_6	5.0	0.12	5.3	0.16	1.8	0.14	2.1	0.011	2.2	0.02
B_1, R, B_6, N.A.	6.4	0.15	4.5	0.13	2.3	0.18	2.8	0.014	2.2	0.02
Yeast	5.5	0.13	2.3	0.07	2.9	0.21	6.2	0.031	4.6	0.05

rats lost almost as much weight as the control rats on the no-yeast diet. On B_1 alone they lost less weight; on B_1 plus riboflavin they did much better; on B_1 plus B_6, still better; on B_1 plus riboflavin plus B_6 they almost maintained their weight; and on all 4 vitamins the rats made a small gain, but their weight still fell far short of that of the yeast control group.

During the first 25 to 30 days the rats offered all 4 vitamins grew at almost the same rate as the normal controls, but after this time they stopped gaining weight. It is not astonishing that the weight gains made by the animals closely parallel the caloric consumption of each group.

Endocrine glands. The weights of all the endocrine glands of the control no-yeast group of rats fell far below those of the control yeast group. The loss of body weight of the rats without any components of the vitamin B complex accounted for a good part of the discrepancies in the endocrine weights of the two groups. However, even when the endocrine weights were calculated in relation to body weight, it was found that the weights of thymus, uterus, ovaries and adrenals still fell below those of the yeast controls. The effects produced on the endocrine glands by

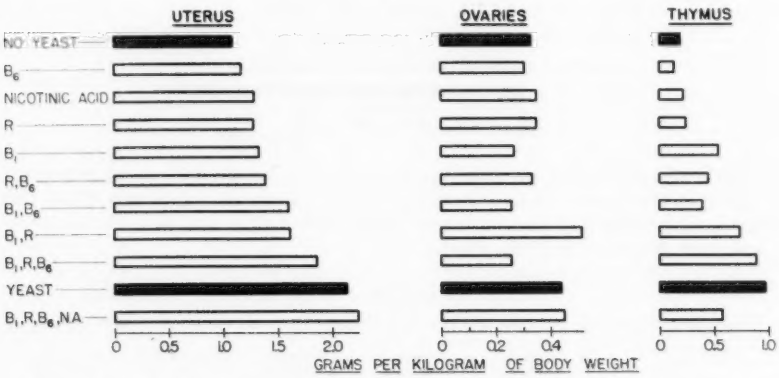

Fig. 3. Graph showing the average weight of the uterus, ovaries and thymus per kilogram body weight for the 2 control (blacked rectangles) and 9 experimental groups of rats.

the various vitamin combinations closely paralleled the effects produced on body weight, caloric intake and the appetite for carbohydrate, fat and protein. Figure 3 summarizes some of the results. It gives the weights of the uterus, ovaries and thymus in grams per kilogram of body weight. For the no-yeast group the uterus averaged 1.1 grams, and for the yeast group, 2.1 grams, or nearly twice as much. The single vitamins had very little effect on the uterus. Here again definite changes depended on combinations which included thiamin chloride. The rats with all 4 components had uteri with weights which averaged slightly above those of the control yeast group (2.2 grams). The ovaries showed similar, however less consistent, effects. The thymus showed the most striking effects. Whereas in the yeast controls the thymus weights averaged only slightly less than 1.0 gram, in the no-yeast controls they averaged only 0.2 gram.

The various combinations of vitamins, especially those including thiamin chloride, progressively increased the thymus weights. The adrenals showed similar effects. However, on the single vitamins, riboflavin and B_6, or on the combination of these two components, the adrenals almost attained the weights of the yeast controls.

The vaginal smear cycles closely paralleled the uterine weights. In the control no-yeast group of rats the vaginal smears showed a dioestrous condition after the 14th day on the diet, while the rats on the full diet had regular 4- to 5-day cycles throughout the 40-day observation period. With all of the single vitamins the regular cycles persisted only about the same length of time as with the no-yeast diet. With the combinations the cycles persisted progressively longer. On all 4 vitamins, 3 rats showed regular cycles throughout the 40-day period; one rat, up to the 33rd day. Clearly, the 4 vitamins maintained the reproductive tract even though they did not fully maintain the entire body.

Discussion. By their appetite, the rats deficient in all components of the vitamin B complex indicated that they were unable to use carbohydrate, and especially protein, but were able to use fat. Biochemical studies have already shown that rats deficient in vitamin B_1 are unable to utilize carbohydrate. The inability to use protein also agrees with the reports of Hartwell (1921, '22, '28), Reader and Drummond (1925), Tscherkes (1926), Cox and Hudson (1929), Bell (1934), and others who showed that a physiological relationship exists between the animal's ability to utilize protein and the amount of vitamin B present in the diet. These results indicated that it is chiefly the number of the then called vitamin B_2 components upon which the protein utilization depends. Our results indicate that thiamin chloride and especially riboflavin play important parts, but that nicotinic acid and B_6 also participate. However, the great discrepancy between the protein appetite of rats which received all 4 of the components and that of rats which received yeast indicated that protein appetite may depend even more on some of the other components, such as pantothenic acid, W factor, adenylic acid, or other ingredients of yeast, such as choline chloride, nucleic acid, etc. The rats further indicated by their appetite that riboflavin may play a part in the utilization of fat. We have not found any biochemical reports which would support this observation.

Thus, from these experiments we see that the higher the number of B vitamins present in the diet the greater is the appetite for carbohydrate and protein and the smaller the appetite for fat, and further, that there exists an inverse relationship between the carbohydrate and fat appetite. Thus far we have invariably found that as one increases the other decreases.

The results also indicated a close dependence of the thymus and the reproductive tract on the vitamin B content of the diet. Here again all

4 components helped to maintain the reproductive tract and the thymus. Singly, if any, they had only a small effect.

The fact that the appetite for calcium and phosphorus solutions increased as more and more vitamins were added may indicate that these vitamins helped the rats to utilize these minerals or they may have only indirectly assisted in growth and maintenance of the skeleton.

Attention may be called again to the fact that under these circumstances, when rats were able to regulate their intake of these various purified substances, in the 40-day observation period no deficiency symptoms appeared. It is possible that in other experiments in which a mixture of regular synthetic or natural food diets was used the appearance of pathological symptoms depended more on harmful effects which resulted from the inability of the animals to increase or decrease their consumption of fat, carbohydrate or protein separately, in relation to the amount of any one component of the B complex eliminated from the diet, rather than from a lack of necessary substances.

SUMMARY

1. A positive control group of 8 young adult rats was given access to our full self-selection diet consisting of: sucrose, olive oil, casein (autoclaved and purified), 5 mineral solutions, cod liver oil and dried baker's yeast. On the selections made these rats gained weight at a normal rate, showed regular 4- to 5-day estrous cycles and normal endocrine glands. Carbohydrate constituted 55.8 per cent of the average diet; fat, 18.4 per cent; and protein, 25.8 per cent.

2. A negative control group of 4 rats was given access to the same substances with the exception of yeast. Thus, their diet lacked all of the B vitamins. They lost weight at once; lost their sex cycle in 14 days; and after 40 days there was a marked atrophy of the endocrine glands, particularly of the thymus, ovaries, uterus and adrenals. Their appetite showed marked changes. They ate little carbohydrate (24.9 per cent), almost no protein (7.5 per cent), and subsisted largely on fat (67.6 per cent).

3. Nine experimental groups of 4 rats each had access to the same basic self-selection diet without yeast, but with crystalline thiamin chloride, riboflavin, nicotinic acid, and vitamin B_6, offered singly or in various combinations. The rats showed an active appetite for each of the vitamins.

4. The ingestion of thiamin chloride particularly stimulated the carbohydrate appetite; riboflavin seemed to have a stimulating effect on the fat appetite; but no one vitamin had an exclusive effect on the appetite for any of the three main foodstuffs. Ingestion of all 4 vitamins increased the carbohydrate appetite almost to normal and decreased the fat

appetite to normal, but failed to increase the protein appetite much past half the normal level; however, the protein appetite was clearly influenced by the intake of thiamin chloride and especially of riboflavin.

5. The ingestion of the 4 vitamins markedly increased the calcium lactate and sodium phosphate appetite.

6. Animals offered all 4 vitamins gained weight for 3 weeks almost at the normal rate; then they lost weight at a moderate rate, but by the end of a 40-day experimental period they had not fallen below their initial weight. The rats on all other combinations of vitamins lost weight steadily. The loss of weight varied almost in inverse proportion to the number of different vitamins. The weight curves closely followed the total caloric intake.

7. The autopsy weights of the uteri, ovaries and thymus indicate that their functioning, too, can be altered by diet. The rats given the 4 vitamin B components came closest to having glands of normal weight (as compared to the yeast controls).

8. Thus, through regulation of the vitamin B components of the diet we were able within large limits to increase or decrease the carbohydrate, fat and protein appetite almost at will. The experiments bring further evidence to show that thiamin chloride seems to serve as a basis for the action of the other components of the vitamin B complex.

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THE INFLUENCE OF STIMULUS STRENGTH AND DURATION ON THE RESPONSES FROM CORTICAL STIMULATION THROUGH IMPLANTED ELECTRODES[1]

SAM L. CLARK AND JAMES W. WARD

From the Department of Anatomy, Vanderbilt University School of Medicine, Nashville, Tennessee

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Moruzzi (1939) has lately studied responses to cortical stimulation in non-narcotized rabbits, though the brain was exposed. Such acute experiments upon man and animals have usually involved exposure of the brain with restraint or recent anesthesia, factors which affect the results. With electrodes permanently implanted in the skull and making contact with the brain it is possible to apply stimuli to the cortex without many of the unnatural factors accompanying acute exploration (Clark and Ward, 1937). It is true that with implanted electrodes only a few points on any one animal can be stimulated, but this factor can be offset by the use of more animals. And there is the extra advantage that in a single animal the same point can be stimulated repeatedly on the same and on successive days under special conditions for a period of days or weeks. Lubinska and Konorski (1939) have seen the advantages of a similar procedure.

Since the beginning of the use of implanted electrodes in this laboratory a number of animals, including cats, monkeys, dogs and goats, have been devoted to such experiments. The observations to be reported here have been taken from the recorded results of the numerous stimuli applied through these electrodes. A 60 cycle sine-wave current was the source of stimulation.

METHOD. The electrodes employed were unipolar. The stigmatic electrode was a piece of silver or platinum wire, sealed in a glass rod of appropriate size with one end rounded off so that the wire was flush with the surface which was to be in contact with the brain. At the other end of the wire a drop of solder furnished a surface for a pressure contact for one lead wire from the stimulating apparatus. The glass-covered wire was inserted into a segment of rubber tubing and this into a stainless steel jacket. The jacket had tapering threads on one end and was screwed into

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a small trephined hole in the skull after the exposed dura mater had been removed. This metal jacket in contact with bone, subcutaneous tissue and skin acted as the indifferent electrode. A special clip allowed the lead wires to be attached and detached rapidly to the electrode.

The stimulating device was similar to one described by Myers (1936) and consisted of a small transformer attached to the 110-volt lighting circuit, with a potentiometer connected so as to allow variation in the strength of the stimulating current and with a voltmeter to record it. A timing device was constructed which allowed duplication of stimuli as to length.

RESULTS. Usually stimulation of animals was delayed until the day following implantation of the electrode, and then to determine a threshold a stimulus thought to be too weak to produce much effect was applied. Following this at intervals of a minute or more slightly stronger stimuli were applied until one was accompanied by definite movement. Record was kept of the duration, strength and time of application of each stimulus, as well as a description of its effects on the animal. The threshold stimulus for a point was arbitrarily chosen as that stimulus which produced visible movement only during the time of its application.

With the apparatus as it was arranged, the average threshold selected for stimulating a point on the motor area was 1 to 2 volts applied for 2 to 4 seconds. Such a threshold remained fairly constant during an experiment in the same animal though it might vary from day to day. On any particular day the threshold could be varied by experimental procedures, and by some physiological factors.

Most stimuli were applied for two seconds (or more) in order that the movements produced would have time to show themselves in completed form during the time of stimulus. For example, the movements most often used in these experiments were batting or digging of the fore paw. These movements, easily obtained from the anterior margin of the cat's motor cortex (Ward and Clark, 1935), were rhythmic, and with them it was possible to observe the repetition of a cycle instead of just a sustained contraction.

After establishing a threshold, experiments were done with variations in the strength and duration of stimuli as well as the interval between successive stimuli. The threshold stimulus was used as a basis for comparison and could be repeated whenever necessary as a check. By raising the voltage or prolonging the stimulus an after discharge could be made to appear. Such after discharges varied all the way from mere prolongation for a few seconds of movement of the affected part of the animal to a complete epileptic attack lasting one or more minutes.

In order to illustrate the effects of stimulation graphic representations of some of the results are shown in accompanying figures 1 and 2. In the

diagrams each horizontal bar represents one stimulus and its effects. The length of the bar to the left of *O* represents volts. The length of bar to the

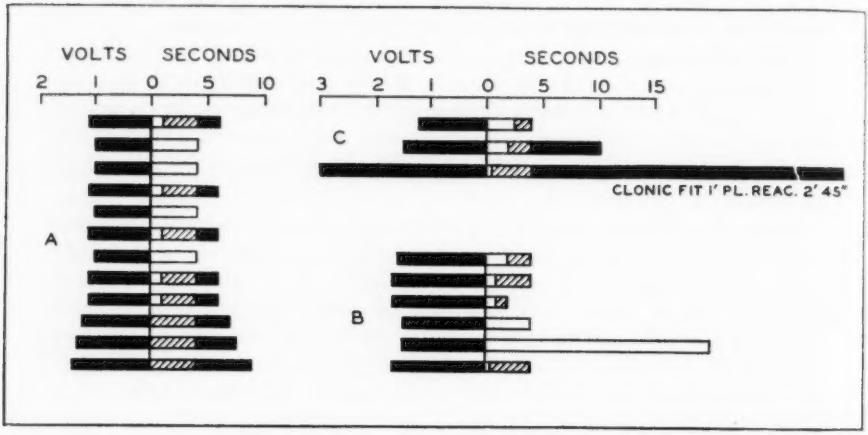

Fig. 1. Graphic representation of a few stimuli and responses to cortical stimulation in two cats. For description see text. In each cat the electrode was on the left anterior sigmoid gyrus, the movement produced being digging with the right fore paw.

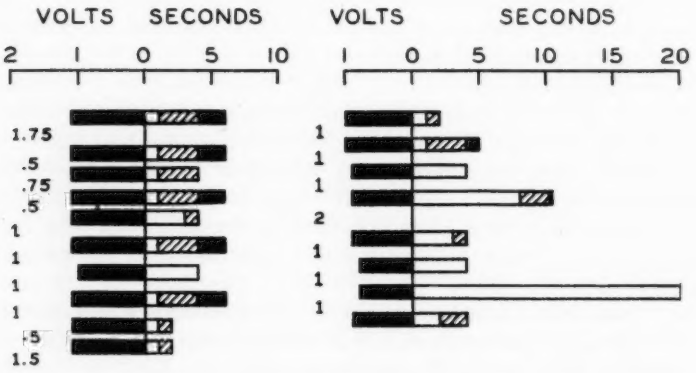

Fig. 2. Graphic representation, described in the text, showing the effect of varying intervals between stimuli on the responses to cortical stimulation in a cat. The electrode and movement were the same as in figure 1A. The numbers in the column to the left represent the intervals in minutes between successive stimuli.

right of *O* represents time. The duration of the stimulus in seconds is represented to the right of *O* in the portion of the bar that is not solid black. When movement appears during the stimulus the bar is filled

with oblique lines. The solid black part of the bar to the right represents the duration of movement following completion of the stimulus, i.e., after discharge. The white part of the bar to the right before the appearance of motion represents the period of latency. The interval between successive stimuli is two minutes, unless otherwise indicated by a number to the left of the spaces between bars.

The effects from stimuli of equal duration but different strengths. When sufficient time for recovery was allowed between successive stimuli the latency of the response and the magnitude and duration of the movements resulting from stimuli of constant length varied with the strength of the stimulus employed.

In figure 1A, it can be seen that a stimulus to the motor area of a cat's brain of 1 volt for 4 seconds gave no response, but 1.1 volts for 4 seconds gave after about 1 second latency, a response which lasted 2 seconds beyond the stimulus. When the stimulus was increased to 1.2, 1.3 and 1.4 volts (the last three bars) the latency was abolished and the after effect prolonged.

The effects from stimuli varied in duration and strength. The responses to stimuli varied with the duration of the stimulus as well as the strength. In figure 1B, (the same electrode as in 1A but on another day) it can be seen that a stimulus of 1.6 volts for 4 seconds gave a response after 2 seconds' latency but with no after effect, while 1.7 volts for 4 seconds gave a response after a shorter latency. The same strength stimulus (1.7 volts) for 2 seconds gave a response with the same latency and no after effect. One and five-tenths volts gave no response with a stimulus of 4 seconds or even after a stimulus 20 seconds in length. Two minutes after the latter stimulus, however, a stimulus of 1.7 volts gave a typical response following a very brief latent period.

Figure 1C shows the results of three stimuli at two minute intervals in another animal. The first stimulus, 1.2 volts for 4 seconds, was followed by a response after a latency of 2.5 seconds. The next stimulus, 1.5 volts for 4 seconds, after a shorter latent period gave a response with a 6 second after effect. The third stimulus, 3 volts for 4 seconds, resulted in a clonic fit lasting a minute, with a loss of placing reactions for a total of $2\frac{3}{4}$ minutes.

The influence of varying the interval between successive stimuli. Figure 2 shows a succession of stimuli through the same electrode that was used in figure 1A and B but with variations in the intervals between stimuli. (The second column follows the first after 1.5 min.) The intervals between stimuli are indicated to the left of each space, but are also proportionately represented by the width of the space between successive bars. It can be seen that a standard response was obtained with a stimulus of 1.1 volts for 4 seconds in which a latency of 1 second and an after effect of 2 seconds accompanied the response, provided at least 0.75 minute elapsed between

stimuli. With a stimulus of 1 volt a lessened response was obtained. With 0.9 volt for 4 seconds no response was obtained after a minute interval, although a 10 second stimulus (at 0.9 volt) gave a response after an 8 second latency with also a slight after effect. A stimulus of 0.9 volt for 4 seconds gave a response when 2 minutes elapsed between stimuli; while a stimulus of 0.8 volt gave no response with 4 seconds' or 20 seconds' stimulation following a one minute interval.

The effects of stimuli of constant strength and duration applied after short equal intervals. While an interval of a minute or more between stimuli was necessary to allow the reproduction of the original effects of successive threshold stimuli (as defined), the application of a stimulus for the first

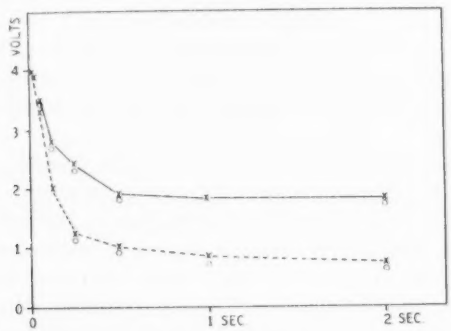

Fig. 3. Graph showing the influence upon threshold strength of altering the duration of the stimulus. The duration of stimuli is expressed in seconds on the horizontal axis, the strength in volts on the vertical. The two curves were obtained from experiments on different cats. The points marked with *X* indicate the stimulus strength and duration necessary for threshold response. The points marked *O* indicate the nearest preceding ineffective stimulus.

two seconds out of every fifteen for several minutes resulted in the establishment of a new level of response. The first response in such a series of stimuli was like that to previous stimuli of threshold strength, but the responses to the next few stimuli were progressively diminished until a constant but lessened response following each stimulus successively applied. Such a series of stimuli could be continued for ten minutes or more without abolishing the response, or appreciably altering its magnitude after the new level was established.

The effects of decreasing the duration and increasing the voltage of successive stimuli. Another type of experiment was done to determine the effect of increasing the voltage but decreasing the duration of successive stimuli in a series (fig. 3). After the establishment of a threshold, a stimulus of threshold strength was applied for half the established time. If the re-

sponse did not appear, the next stimulus was increased in strength by 0.1 volt and this process repeated with successive stimuli until a response did appear. Then the duration of the following stimulus was arbitrarily cut in half and so on. With brief stimuli it was not possible to produce responses of sufficient duration to be described as entirely similar to those following stimuli several seconds in length, but it was possible to see evidence of responses, even though they were mere twitches of the affected muscles; and it was evident that the strength of each new threshold stimulus increased as its time of action was decreased.

Discussion. From examination of the figures showing samples of series of stimuli to the cerebral cortex through implanted electrodes it can be seen that the effects of successive applications of a given stimulus of near threshold value were reasonably constant when sufficient time between stimuli was allowed for recovery.

If a stimulus was applied which was increased beyond the previously established threshold by raising the voltage or lengthening the time of application, until an after discharge appeared, then the time of recovery was prolonged. Such an after effect was constant in its duration for the particular stimulus which provoked it, and could be repeated subsequently when the conditions were the same. The duration of after effect depended upon the amount of increase in stimulus strength or duration, or both, over that of the selected threshold as well as upon the immediately preceding history of the experiment. With only slight increase of stimulus above threshold strength the magnitude of the muscular response was greater; if the response was rhythmic the rate of the rhythm might be quickened. At a strength of stimulus two or three times that of threshold the animal would be thrown into a generalized epileptic seizure lasting several minutes, which spread in its beginning like the "march" of a Jacksonian convulsion. If a generalized epileptic attack was provoked phenomena associated with it might be present for five to ten minutes or more and recovery was much delayed. During this period of recovery stimuli of threshold value would fail to produce the usual response, and the stimulus ordinarily sufficient to provoke a fit might fail entirely, or be followed by an abbreviated after discharge, the duration of which varied with the time since the preceding convulsion.

The same type of response as that following threshold stimulus was produced by a slight lowering of the voltage and appropriate lengthening of its time of action, but in this case the movements often appeared a short while after the beginning of the stimulus. The response was therefore preceded by a latent period. The length of this latent period has been shown by Ward (1938) to vary for a given stimulus dependent upon such factors as: the position of the animal's head, the position of the responding part; the just preceding sensory experience of the animal; its

state of wakefulness; the integrity of reflex paths, etc. Lubinska and Konorski (1939) demonstrated similar alterations in the latency in varying physiological states.

If the strength of successive stimuli was progressively lowered by small amounts below that of the threshold, it was necessary at each level tested to prolong the duration in proportion to produce visible responses. Not far below threshold a limit was reached at which point no response could be obtained with a stimulus of indefinite duration (figs. 1B and 2). The division between an effective and an ineffective stimulus was rather sharp. With a prolonged weak but effective stimulus, say of 15 to 20 seconds' duration, the visible movements often ceased before the end of the stimulus.

After cessation of visible movements provoked by a given stimulus the placing reactions in an affected limb might be negative for a while longer. Furthermore, following a stimulus too weak to produce visible movement even after 20 to 30 seconds' application, the placing reactions of the proper extremity might be abolished for a while. This may be correlated with the observations of Moruzzi on the evidence of bioelectric effects from the cortex following subliminal stimuli.

As the stimuli in our experiments were relatively long in duration we were constantly dealing with the phenomenon of facilitation, which has been studied repeatedly. This is illustrated, for example, by the period of latency preceding responses to subthreshold stimuli, the appearance of a response some time after the onset of the repetitive stimulus being one evidence of facilitation. Such a latency was termed by Cooper and Denny-Brown (1927) "summation period" to distinguish it from the latent period of nerve fibers. These authors state that facilitation may be avoided by spacing stimuli one minute apart. In the present experiments it was the rule to allow two minutes to elapse between stimulations, unless a briefer time was indicated as a part of the experiment.

In these experiments we have also been aware of the factor for extinction described by Dusser de Barenne and McCulloch (1937). They observed that the optimum interval for extinction in the monkey in the waking state was 4 seconds, and that it was prolonged by anesthesia. It is possible that the conditions of our experiments are sufficiently different to result in different manifestations of this phenomenon. The diminished responses obtained from the stimulation after the first of a series when the stimuli were applied for the first 2 seconds out of each 15 are apparently evidence of the operation of this factor. It might be questioned whether the diminished response to stimulation in the longer period following an epileptic seizure is evidence of a more prolonged operation of the factor for extinction, but the time limits of the chart of the above authors (1939, fig. 15) might allow this in part. During the period immediately follow-

ing an induced epileptic seizure, when the placing reactions are negative it is obvious that the cortex is not functioning normally. We have pointed out (1938) how this temporary state resembles the permanent condition produced by removal of the motor area. Even after the placing reactions have returned, however, for a while stimulation of the same point on the cortex with the same stimulus which just previously provoked an epileptic seizure will either not produce a fit, or will produce one of less severity. This state of diminished response to strong stimulation may last several hours.

The actual values of the stimuli of threshold strength are no doubt not indicated by the figures recording the voltages at the ends of the electrodes we have used, since the electrodes rest on the pia mater in a bath of cerebrospinal fluid; and in addition to the pia there is the superstructure of the cortex between the stigmatic electrode and the cortical elements (of the deeper layers, Dusser de Barenne, 1934) which are activated. However, relative strengths of successive stimuli are indicated and the stimulation is measurable in terms which can be applied to other conditions. The same apparatus has been used by Dr. Cobb Pilcher in exploring exposed human brains and similar threshold values obtained, though with anesthesia thresholds were higher. This similarity of thresholds in different species recalls the experiment in which Leyton and Sherrington (1917) connected the exposed brains of a cat, a macaque, and a chimpanzee in series with a stimulator and found that motor responses occurred at approximately the same threshold in each.

We wish to acknowledge the aid in conducting some of the experiments on which this paper is based of Mr. J. Thomas Payne and Mr. Fred C. Cowden.

SUMMARY

Cortical stimulation through implanted electrodes allows the study of responses in unanesthetized and unrestrained animals, in which with constant conditions responses to stimuli can be repeated and predicted. With stimuli of near threshold strength the response varies quantitatively with the duration and strength of the stimulus. An interval of one minute or more is necessary for recovery from the effects of a stimulus of near threshold strength.

Subthreshold stimuli followed by no visible movement may be followed by a temporary loss of placing reactions in the appropriate contralateral limb.

The duration of responses beyond the time of stimulus allows the production of after effects up to and including complete epileptic seizures.

The duration of such after discharges is predictable when the factors for variation are properly considered. The recovery period is prolonged following such after effects.

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THE GRADATION OF THE INTENSITY OF INSPIRATORY CONTRACTIONS[1, 2]

ROBERT GESELL, A. KEARNEY ATKINSON AND RICHARD C. BROWN

From the Department of Physiology, University of Michigan, Ann Arbor

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It has been generally agreed that the mechanical energy employed in eupneic breathing is provided mostly, and sometimes entirely, by the inspiratory muscles. Similar conclusions are now warranted for the hyperpnea of oxygen want for if the expiratory muscles are active during eupnea their contractions are often seen to weaken or actually to disappear as pulmonary ventilation increases (Brown, Atkinson and Gesell, 1939). Thus the burden of breathing may fall entirely to the inspiratory muscles even during conditions of respiratory distress. Not only must these muscles provide greater energy to overcome the greater distortion of the torso and lungs during each inspiratory act but they must supply increasing potential energy for the expulsion of the air as well. The adjustment of inspiratory contraction thus promises to be the most important single factor of respiratory control. Our present paper deals specifically with the basic nature of the graded adjustment of these contractions to the mechanical requirements of the inspiratory act and with the sudden relaxation of their activity during the expiratory period (see fig. 1 and legend).

Two points are already clear from the studies of Adrian and Bronk (1928). Strength of muscular contraction is determined primarily by two factors: 1, the frequency of muscle fiber twitch, and 2, the number of active muscle units engaged. Our problem then resolves itself into specific phases. First, the relative importance of twitch frequency and of the number of units participating, for that may suggest the underlying integrative mechanism. Second, the precise manner in which these basic tools of gradation are used, and third, a hypothetical consideration of the nervous mechanism effecting this use.

Two obvious assumptions may be made. Mechanical energy may be liberated in a suddenly developing rectangular block sufficient to accomplish the required inspiration of air in the time allotted to the inspiratory act. With that method inspiration would tend to be jerky. Or

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[2] This study was supported by a grant from the Rockefeller Foundation.

mechanical energy may be liberated to match the growing requirements of increasing resistance to pulmonary inflation. That method should produce a smoother inspiration.

In their studies on the action potentials of the phrenic nerve in the rabbit, Adrian and Bronk saw no indications of increasing strength of contraction as each *individual* inspiration developed. They state that

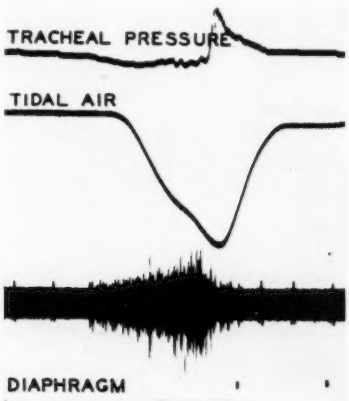

Fig. 1. An inspiratory fusillade recorded with widely separated floating electrodes placed on the surface of the diaphragm of the dog. The upper record shows changes in tracheal pressure during the inspiratory and expiratory phase. The middle or tidal air record shows the velocity of movement of the inspired and expired air. Due to the weight of the spirometer there is a large time lag. The peak of the electrogram and the trough of the tidal air tracing should coincide.

The relatively even descent of the spirometer tracing and the relatively constant inspiratory pressure indicate that the air is inspired at a relatively even velocity despite a continuously increasing resistance to the expansion of the lungs. The rising electrogram indicates a progressively increasing strength of inspiratory contraction. The abrupt emptying of the lungs, indicated by the sudden rise of the tidal air record and the sudden development of positive intratracheal pressure, the "expiratory puff," shows the suddenness of the expiratory act. The sudden relaxation of the inspiratory muscles revealed in the electrogram conforms with a powerful passive expiration.

each nerve fiber discharges at uniform frequencies throughout each inspiration (between 20 to 30 per sec.) and that accession of new muscle units is missing. Thus strength of diaphragmatic contraction remains uniform throughout inspiration. They "looked for evidence of accession of fresh fibers as contraction develops by comparing the duration of the single fiber discharge with that of the whole nerve," but "found no clear evidence of single fiber discharge being any shorter than that of the whole nerve."

Forcible contractions produced by clamping the trachea yielded twitch frequencies of 50 to 80 per second which led these authors to conclude that "variations in frequency of discharge between the limits of 20 to 80 a second are excellently adapted for producing contractions of graded intensity without bringing fresh fibers into play."

The results of Adrian and Bronk (1929) on the flexion reflex were very much the same as those on the diaphragm. They state "that grading of the contraction appears to be due mainly to this change in frequency, for there is little evidence of changes in the number of neurons in action." In the extension reflex on the other hand they found "that many fresh neurons come into play as contraction develops." Bronk and Ferguson (1935), reporting on the internal intercostal muscles, conclude that "Variations in depth of intercostal respiration are a result of variations in frequency of discharge from the individual nerve cells, the duration of their discharge and the number of nerve cells in action." These results are similar to those of Gesell (1936) and Gesell and White (1937) on the internal and external intercostal muscles in which a slowly augmenting triangular pattern of activity was described. Rijlant (1937) is of the same opinion as Adrian and Bronk, that the contractions of the diaphragm are of the explosive type in the cat, rabbit and the dog.

Granting the validity of these results, one is confronted with the query—why should the gradation of contraction of inspiratory muscles differ in such important details? Why should the relatively unimportant intercostal muscles deliver a nicely graded contraction capable of overcoming a progressively increasing resistance, and the more important diaphragm deliver energy in an explosive rectangular block? Since the activity patterns of respiratory contractions reveal the details of the end effect of the nervous integration of the respiratory act, and tell us in such precise form what we wish to explain, it seemed most desirable to extend the existing studies on the nature of respiratory contractions.

Results. On moving highly selective electrodes indiscriminately from one point to another, one soon finds with the aid of a loud speaker alone, that many fibers are inactive during eupnea, that some fibers twitch throughout the entire period of inspiration while others twitch but once or twice at the very end of inspiration, that the remaining active fibers contract variable periods of time and fill in the intervening gap, that twitching of any muscle unit begins with a relatively low frequency and accelerates as inspiration progresses and that cessation of twitching is relatively abrupt. Due to the differences of sound (as heard with the loud speaker) produced by individual muscle fibers, it is possible to distinguish one series of twitches superimposed upon another in every stage of the inspiratory act. Such results are common to the internal and external intercostal muscles and the diaphragm, thus indicating that ac-

celeration of frequency of twitch and recruitment of new units are *universally* employed in the gradation of inspiratory contractions.

Widely separated, instead of closely approximated electrodes, will pick up simultaneously the potentials of individual muscle units in every stage of recruitment such as illustrated in records 1, 2, 3, 4, 5 and 6 of figure 3 and combine them into a heterogeneous fusillade. No single set of discrete

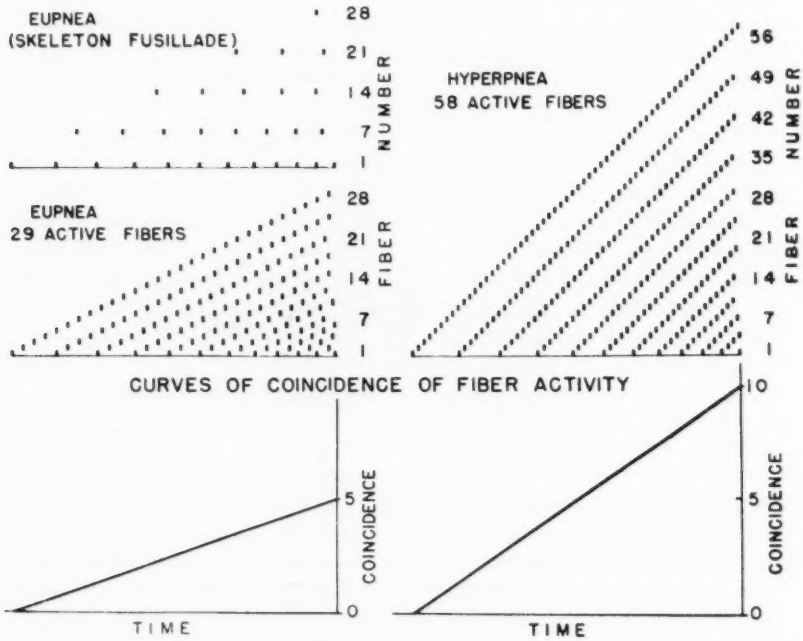

Fig. 2. A schematic representation of the use of recruitment and acceleration of muscle fiber twitch in the adjustment of inspiratory contractions to the mechanical requirements of shallow and deep breathing. For explanation see text. If inspiration is sufficiently strong and prolonged the initially active fibers may reach their maximum twitch frequency an appreciable interval before the termination of the fusillade (see fig. 3).

potentials of any single muscle unit can be heard but rather a mixture of asynchronous potentials of slowly increasing and suddenly decreasing volume, agreeing with the graphic record of the diaphragmatic fusillade of figure 1.

The necessarily increasing incidence of potentials as one unit after another is recruited we believe explains the increasing volume of sound and the triangular shape of the electrical fusillade in figure 1. This point is

schematically illustrated in figure 2, where 29 muscle units were arbitrarily chosen as participating in the hypothetical eupneic fusillade at the left. Muscle unit no. 1, at the bottom of the schema, is the first to contract. It goes through its normal course of acceleration from left to right. Unit

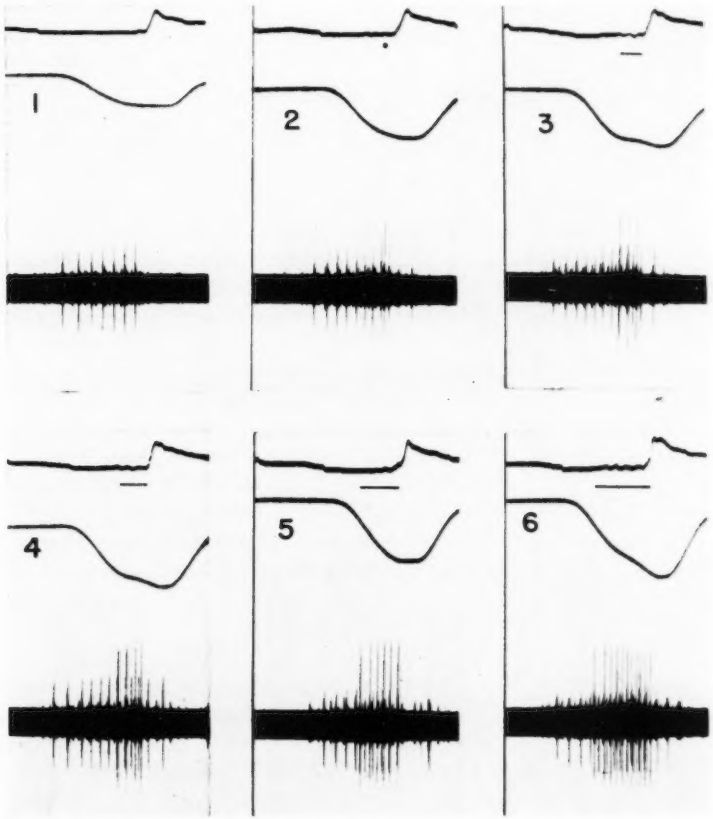

Fig. 3. Actual records of the activity of two muscle units of the internal intercostal muscle during a progressively increasing chemical stimulation resulting from rebreathing into a confined space. Acceleration of twitch frequency, recruitment, inspiratory encroachment, and increasing twitch number are illustrated (see text).

no. 2 contracts next, and unit no. 3 follows closely on unit no. 2, etc. Each muscle unit begins its activity at the minimum frequency of twitch and accelerates, in this schema, at the standard pace up to the end of inspiration. The muscle units which are recruited late in the phase of inspira-

tion, therefore, fail to acquire the final high frequency of twitch of unit no. 1. This is more clearly seen in the upper skeleton fusillade in which units 1, 7, 14, 21 and 28 are shown by themselves. On moving a vertical line along the complete fusillade, the incidence of potentials will be seen to increase from left to right as more active units are recruited and as the frequency of twitch of each unit increases. One potential thus adds to the other and raises the electrical record as already noted in figure 1. The photographic record rises, unlike the smoothed schematic curve of incidence of fiber activity in figure 2, in a serrated fashion, agreeing with an actual varying incidence of overlapping potentials which must occur in any purely asynchronous mechanism such as muscular contraction. We have been unable to find groups of synchronized activity of muscle units corresponding to the frequency of twitch such as described by Adrian and Bronk (1928) and Rijlant (1937). On that point we are in agreement with Wyss (1939). Since each muscle fiber twitch adds its quota of mechanical energy to that of other contracting fibers, as it adds its incident potentials, the rising shadow of the electrical fusillade becomes an index of the strength of muscular contraction.

In addition to the automatic increase of intensity of each eupneic inspiratory contraction necessary to overcome the physical resistance to breathing under uniform respiratory demands, there must also be a means of controlling the depth of inspiration to meet augmented demands such as oxygen lack or carbon dioxide excess. As figure 3 shows, the gradation of inspiratory contractions meeting an increasing chemical stimulation is no different in principle from that employed in meeting increasing physical resistance during each inspiratory act. In this experiment the dog was made to rebreathe a restricted volume of air. As records 1, 2, 3, 4, 5 and 6 show, there is an orderly change in the details of contraction as the chemical stimulation mounts, and the depth of breathing increases. Muscle unit no. 1 begins with 7 twitches in respiration no. 1. In breath 2 the number of twitches is increased to 9. In breaths 3, 4, 5 and 6 the twitches have reached a maximum steady number of 12. In the mean time fiber no. 2 has begun to contract, with but a single twitch at the end of inspiration no. 2. In breaths 3, 4 and 5 it twitches 3, 4 and 6 times respectively, and in breath 6 there are 11 twitches in all. As the number of twitches increases, activity begins earlier and earlier in the inspiratory phase. This advancement of activity is indicated in figure 3 by the horizontal line under the tracheal pressure record.

The schematic fusillades of figure 2 will indicate more clearly the mechanisms underlying the change from eupnea to hyperpnea. Twenty-nine additional active muscle units recruited into activity by a hypothetical increased chemical stimulation, have been added to the original 29 units of the hypothetical eupneic fusillade. Initial activity of succeeding units,

therefore, follows in more rapid succession and thus increases the strength of inspiration at a greater pace than normal. The units initially active during eupneic breathing now contract a greater number of times per inspiration and, therefore, contribute an increased amount of mechanical energy to the inspiratory act, thus units 7, 14, 21 and 28 instead of contracting 8, 5, 3 and 1 times respectively now contract 10, 8, 6 and 5 times. The newly recruited units 30 to 56 now top off the contraction. A count of the total number of twitches for the complete fusillades shows 150 for the eupneic inspiration and 282 for the hyperpnea. Doubling the number of fibers, therefore, falls a little short of doubling the sum total of twitches. That is due to the fact that a larger number of fibers fail to reach a high frequency of twitch during hyperpnea.

The advancement of activity into the earlier stages of inspiration as chemical stimulation increases is most rapid in each new unit immediately after its recruitment into service; see for example figure 4 in which the recorded potentials of three muscle fibers during a progressive rebreathing hyperpnea were transposed under a single respiratory schema. When unit no. 1 first recorded it was already twitching 5 times per inspiration and consequently the initial twitch of this series had advanced well into the earlier stages of inspiration. But as hyperpnea increased initial activity encroached still more upon the early phase of contraction. Finally each twitch occurred at the approximate onset of inspiration. Unit no. 2 fortunately recruited with a single twitch and, therefore, shows the initial rapid "inspiratory encroachment." The lower curve shows the inspiratory encroachment of initial activity of unit no. 3.

If the energy liberated by a single twitch of a single muscle fiber is tentatively considered as the unit of muscular energy, the number of such energy units contributed by individual muscle fibers in the course of an increasing ventilation from increased chemical stimulation is worthy of consideration. It is readily seen in figure 4 that as chemical stimulation increases, each muscle fiber contributes an increasing number of twitches to each inspiration. That is due to a prolongation of the period of twitching and to an acceleration of the frequency of twitch. The course which this increase commonly follows is shown in figure 5. The number of twitches per inspiration of three individual muscle fibers plotted against intensity of stimulation (breath number after the beginning of rebreathing) on the abscissae is seen to increase rapidly immediately after each muscle fiber is recruited into activity, then more and more slowly and finally it tends to strike a relatively uniform level though often this level is not fully attained under the conditions of our experiments. The curve is very similar to that already described for inspiratory encroachment in figure 4. A significant fact is that the curve of twitch number appears to have a similar contour at all intensities of chemical stimulation and that newly activated

fibers continue to be successively recruited after the early activated units have already reached or approximated their maximum activity. In

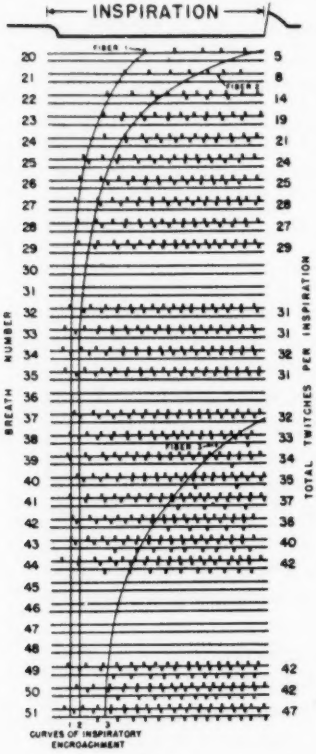

Fig. 4

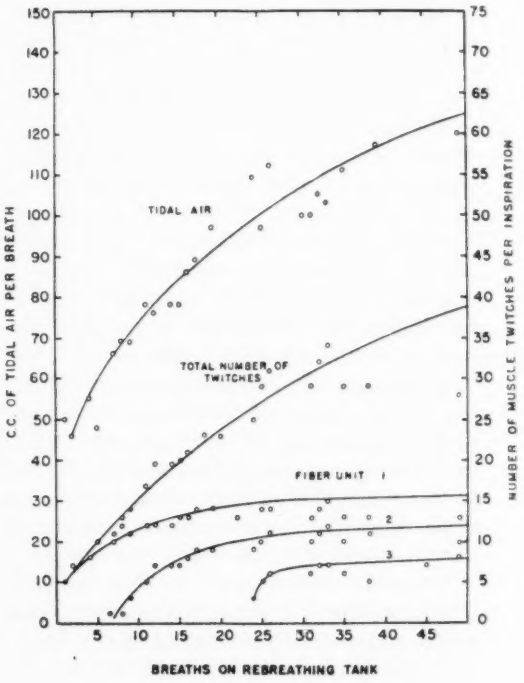

Fig. 5

Fig. 4. A transposition of electrograms of three muscle units under one inspiratory schema during an increasing hyperpnea (breaths 20 to 51 after the beginning of rebreathing into a confined space). The curves show the course of inspiratory encroachment of the initial twitch of three muscle units. The individual records show the gradual increase of twitch number and twitch frequency with increasing chemical stimulation.

Fig. 5. The lower three curves show the relation of the number of twitches per inspiration for three muscle units during an increasing chemical stimulation. The figures on the abscissae indicate the breath number after the beginning of rebreathing into a confined space. Note the lack of agreement of these curves with that of tidal air or strength of inspiratory contraction. There is better agreement between the tidal air curve and that of the total number of twitches.

figure 5 muscle unit no. 3 begins its activity when unit no. 1 is nearly at its maximum response. Obviously the number of twitches contributed by

any *single* muscle unit cannot be regarded as an index of total muscle activity.

Changes in the maximum twitch frequency, that is, the highest frequency occurring during the course of inspiration, are illustrated in figure 6. As was seen for the curves of inspiratory encroachment and of twitch number the curve of twitch frequency in each individual fiber rises rapidly immediately after its recruitment and increasingly slower thereafter, and, as was true for inspiratory encroachment and twitch number, new muscle units

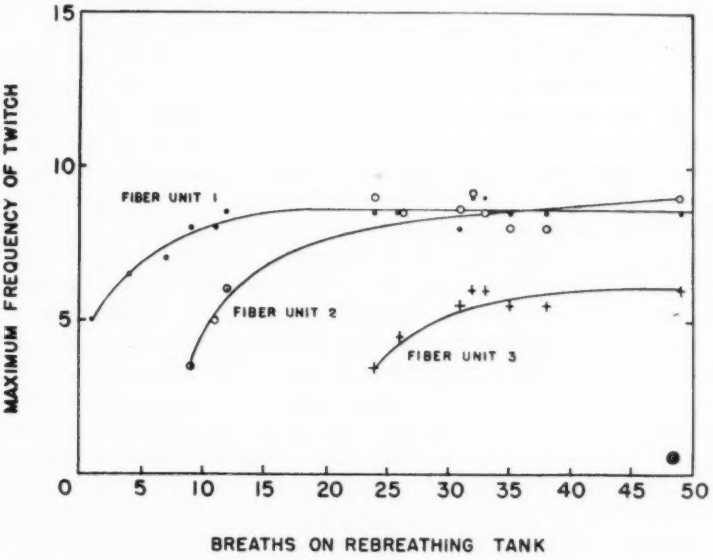

Fig. 6. Maximum twitch frequency of three muscle units during a progressively increasing chemical stimulation. These results and those of figure 5 were obtained from the same observation. Note the low frequency of twitch.

successively recruited run through their complete frequency change after earlier activated units have already reached their maximum frequency of twitch. Maximum twitch frequency of any single unit cannot, therefore, represent the intensity of the contraction of the muscle as a whole as originally suggested by Adrian and Bronk. Because the curves of inspiratory encroachment, of twitch number, and of twitch frequency all have the same shape at all stages of intensity of contraction none can indicate the change of intensity of contraction. We must, therefore, look for another index.

The most important index is in all probability the sum total of twitches or mechanical energy units liberated by all those fibers participating in

contraction. That is suggested by the comparison of the curves in figures 5 and 6. Only when the sum total of twitches of all three units is plotted against intensity of chemical stimulation is there a semblance of agreement with strength of inspiratory contraction as represented by the curve of tidal air (see fig. 5).

It is true that tetanic contraction of a muscle may quadruple the tension developed by a single twitch. This has been noted by Creed, Denny-Brown, Eccles, Liddell and Sherrington (1932) and by Cooper and Eccles (1930) in skeletal muscle and by Adrian and Bronk in the negative intratracheal pressures developed by the contraction of the diaphragm. Yet none of these findings are contrary to the concept that the sum total of individual fiber twitches is the factor determining the strength of contraction, for the total number of individual twitches would vary directly as the frequency of stimulation. Furthermore a control of strength of contraction based purely on a frequency mechanism would yield but a small degree of gradation as compared with the possibilities of recruitment. For instance, there can be no question of the fact that fewer and fewer of the muscle units of the diaphragm contract as an animal is made progressively more hypocapnic. Conversely, it is reasonable to assume that all units are ultimately called into activity by an intense hypercapnia. Admitting that the diaphragm has thousands of individual muscle units capable of the finest gradation of recruitment, it follows that the possibilities of the gradation of intensity of contraction through the mechanism of recruitment may be thousands of times greater than that of gradation by frequency alone. To one accustomed to hearing the amplified potentials of respiratory contractions there can be no doubt of the validity of this conclusion.

Frequency of twitch remains surprisingly low even during strong chemical stimulation, from 10 to 30 in the intercostals and somewhat higher in the diaphragm. Such an arrangement combined with a smoothly graded recruitment to call in extra energy as it is needed must be a most effective protection against fatigue, not only in the muscle, but throughout the central nervous system as well. No single unit is allowed to set a devastating pace leading to an early collapse. Instead new units are called in to support those already contracting. For these reasons we questioned the principle of alternate rest and activity of individual units. And to test this idea more critically we have attempted to learn how long a single muscle fiber would contract without retiring in favor of another. Such periods may run four hours or more. On the basis of such findings one is tempted to suggest that certain muscle fibers, for example those activated by cell 1 of figure 8, are destined to discharge with each inspiration throughout life, during each inspiratory act, whereas those connected with the underlying cells are destined to lead a relatively inactive existence con-

tracting only under emergencies requiring more powerful inspiratory contractions.

It, therefore, seems most significant in this connection that the increase in tension developed by increasing frequency of stimulation of muscle is greatest in the lower twitch frequencies. According to the curves of Adrian and Bronk (1928) and of Cooper and Eccles (1930) the relation is roughly linear in the twitch frequency range normal to each individual muscle. At higher frequencies the strength of contraction is increased relatively

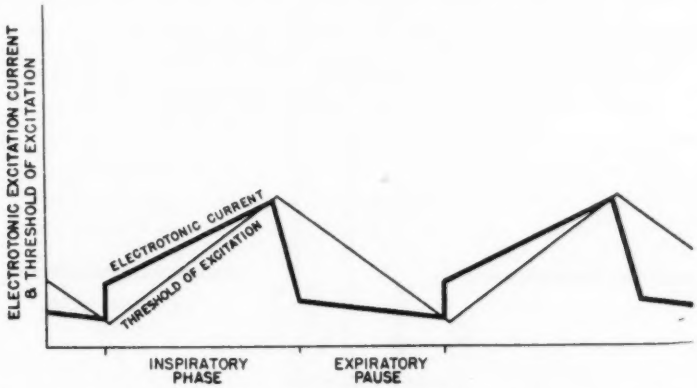

Fig. 7. An hypothetical schema of acceleration and interruption of nerve cell discharge based on the augmenting action of signals returning by the recurrent collaterals.

When the nerve cell begins to discharge the electronic excitation current is conceived to rise due to the progressively increasing number of recurrent signals. The resulting increasing frequency of discharge exhausts the cell and the threshold of excitation of the axon hillock rises. When it is greater than the excitation current the cell stops firing. The electrotonic excitation current then falls abruptly due to the absence of recurrent signals and ultimately more slowly. As the cell recovers during this period of inactivity the threshold of excitation likewise falls and in time the cell fires once more.

little. Thus it should follow that recruitment of one fiber twitch after another in an augmenting contraction adds a relatively uniform amount of energy.

It is well to add that we have attempted to emphasize principles rather than details. Nevertheless completeness requires a brief mention of some variations of response. In certain observations, for example, the curves of twitch number and maximum frequency rose less abruptly at the outset than those described. Some curves rose after the break at a somewhat steeper pitch than did others. We gained the impression that such dif-

ferences held more for the diaphragm than for the intercostals although the results on these two sets of muscles were in general the same.[3] In some experiments it was noted that the curve of twitch frequency of the diaphragm rose longer and broke higher than that of the intercostals. There is some evidence that the machine-like regularity of performance

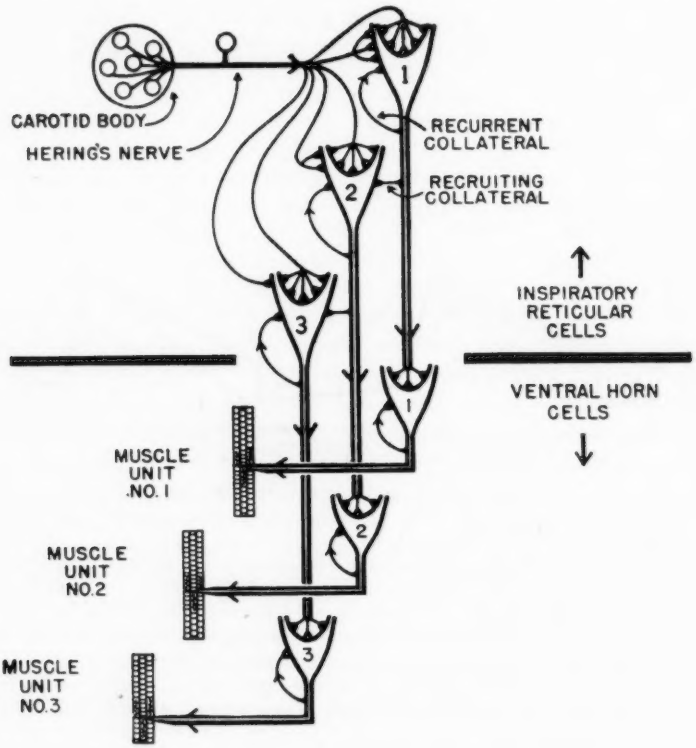

Fig. 8. An hypothetical neuro architectural arrangement of nerve cells designed as a possible explanation of an orderly recruitment of nerve cell activity out of the subliminal fringe.

of the inspiratory muscles is modified by proprioceptive impulses for when asphyxia was produced by clamping of the trachea instead of by rebreathing the curves rose in a different manner. Such difference is probably the

[3] It should be noted that our observations on the diaphragm were made during pneumothorax in which conditions are decidedly abnormal.

result of the addition of one respiratory drive to another. Since our conclusions are based on a large number of findings we have no reason to believe that such variations as do occur contradict the general principles of gradation of contraction set forth above under increasing chemical drive.

THEORETICAL. The rhythmical recurrence of the slowly augmenting pattern of discharge of the inspiratory center during curari paralysis and the complete abolition of periodic afferent signals leads to the conclusion that such activity arises from steady drives acting upon some mechanism peculiar to the inspiratory center (Gesell, Atkinson and Brown, 1940). The regular uninterrupted series of discharges produced by a steady polarizing current in the iron nerve model of Lillie (1923), the Nitella (Osterhout and Hill, 1930), the heart, and the isolated nerve fiber (Adrian, 1930) lend support to an electrotonic theory of nerve cell activity (Gesell, 1939a, 1939b, 1940). The rhythmical interruption of the discharges of an isolated nerve responding to its own current of injury (Adrian, 1930) signifies that secondary as well as primary rhythms may develop in exceedingly simple structures. Our theory of a single possible mechanism of a slowly augmenting nerve cell discharge in brief is this. An electrotonic current flowing in the cell from the dendrites to the axon hillock discharges the cell at its point of exit. This current, assumed to arise from an inherent metabolic gradient and local negativities established by impinging signals tends to fire the cell at a uniform frequency. Due however to signals returning by recurrent collaterals the cell reëxcites itself progressively to increasing activity until it suddenly fails from temporary exhaustion. During the resulting period of inactivity the cell again recovers its normal excitability to its own electrotonic current and then repeats the cycle as suggested in figure 7.

Recruitment of new active units would in turn depend upon simple motor interconnections (fig. 8), between the inspiratory reticular cells comparable to those originally described by Retzius and Linnhosek (cited by Cajal, 1909). According to our hypothetical schema, the activity of one cell is transmitted to a second resting cell, thereby bringing that cell into activity. Acceleration of frequency of discharge and recruitment thus become most intimately related. Cell 1, possessing the lowest threshold of excitation, sets off the inspiratory discharge. It at once dispatches signals to cell 2 (whose excitation current is just below threshold) and momentarily sets this cell discharging. As cell 1 accelerates, it in turn tends to accelerate cell 2. Cell 2 in turn connected with cell 3 tends to activate that cell accordingly. When cell 1 ceases to fire it withdraws its signals from cell 2. Thus all underlying cells cease firing. Since cell 1 ceases abruptly all underlying cells stop discharging in a like manner which explains the sudden weakening of the inspiratory fusillade.

SUMMARY AND CONCLUSIONS

The relatively great importance of the inspiratory act in the mechanics and in the nervous integration of breathing called for a further study of the mode of its gradation.

Since inspiratory contractions produce a progressively increasing distortion of the lungs and torso the amount of energy required to continue the act must vary with the degree of pulmonary inflation.

This energy is found to be supplied in a smoothly graded manner suitable for meeting the increasing mechanical requirements as the lungs expand.

The adjustment of energy is accomplished in two ways, by a progressive addition of newly activated units to the initially weak contraction and by an increased frequency of twitch of all participating fibers as inspiration advances.

Each newly recruited unit begins contracting at its minimum twitch frequence which accelerates as inspiration progresses. Those units recruited early in the phase of inspiration, therefore, attain the highest frequency and deliver the greatest number of twitches.

The gradation of inspiratory contractions during progressive hyperpnea from oxygen lack or carbon dioxide excess is in principle the same as that employed to meet the ordinary increasing mechanical requirements of a eupneic inspiration. Increased depth of inspiration is attained by a combination of recruitment of new muscle units and an intensified activity of those units already engaged. As chemical stimulation increases, initial activity of individual muscle units moves up into the early phases of inspiration thus increasing their number of twitches and their maximum twitch frequency.

The energy liberated by a single twitch of a single muscle fiber is adopted as the unit of mechanical energy of muscular contraction and the sum total of the individual twitches of all participating fibers is regarded as the main factor determining the strength or depth of inspiration. The number of twitches and the frequency of twitch of individual muscle units is not necessarily an indication of the strength of contraction of the muscle as a whole.

Central integrative mechanisms of periodicity and of gradation of the activity of the inspiratory half center are proposed.

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THE DUAL EXCITATORY ACTION OF THE VAGAL STRETCH REFLEX[1]

ROBERT GESELL AND CARL MOYER

From the Department of Physiology, University of Michigan, Ann Arbor

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The emphasis on the inspiratory inhibitory action of the pulmonary vagi has been characteristic of virtually all modern views of respiratory control (Hering and Breuer, 1868; Gad, 1880; Head, 1889, and Adrian, 1933). To us, however, it has seemed more consistent to regard the vagal stretch reflex as an excitatory phenomenon, both inspiratory and expiratory, in which the associated inhibitions of the opposing centers are but secondary manifestations of the excitatory action (Gesell, 1939). The simple experiments here described present evidence supporting such hypothesis.

METHOD. A dog, anesthetized with morphine and urethane, is connected with a rebreathing tank arranged for the absorption of expired carbon dioxide and for the registration of tidal air excursions. The spirometer employed in these experiments is specially constructed to allow an artificial increase of intrapulmonary pressure while recording pulmonary ventilation. Two encircling bands placed around the depilated torso register the changes in circumference at the mid thoracic and mid abdominal levels (Gesell and Moyer, 1935). Segmental and combined respiratory response are then recorded on smoked paper during normal barometric conditions, during increased intrapulmonary pressure and during increased intrapulmonary pressure plus double vagal block. Downstroke in the respiratory records corresponds to an increasing circumference of the chest and abdomen and to a filling of the lungs.

RESULTS. The general trend of results is illustrated in figure 1. In this observation normal breathing is recorded for a period of thirty seconds, after which the lungs are inflated above normal volume by loading the spirometer. During the period of pulmonary inflation which follows, the cervical vagus nerves are twice blocked and deblocked at separated intervals and finally the spirometer is unloaded and the animal allowed to breathe normally once more. The first noticeable effect of the expanded condition of the lungs and torso is an immediate cessation of rhythmic breathing. Since this well-known response is absent or almost missing

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when the vagus nerves are blocked or severed (note the acceleration of breathing during both vagal blocks), it is generally regarded as due to an inspiratory inhibitory action arising in the vagal stretch reflex.

The next visible respiratory response, not as well known as the reflex stoppage of breathing, is a slowly developing expiratory activity. This effect was emphasized by Hering and Breuer when they found a slowly rising intratracheal pressure following an artificially increased pulmonary volume. In our experiments increased expiratory activity is evidenced by a decreased circumference of the abdomen opposing the distending pressure.

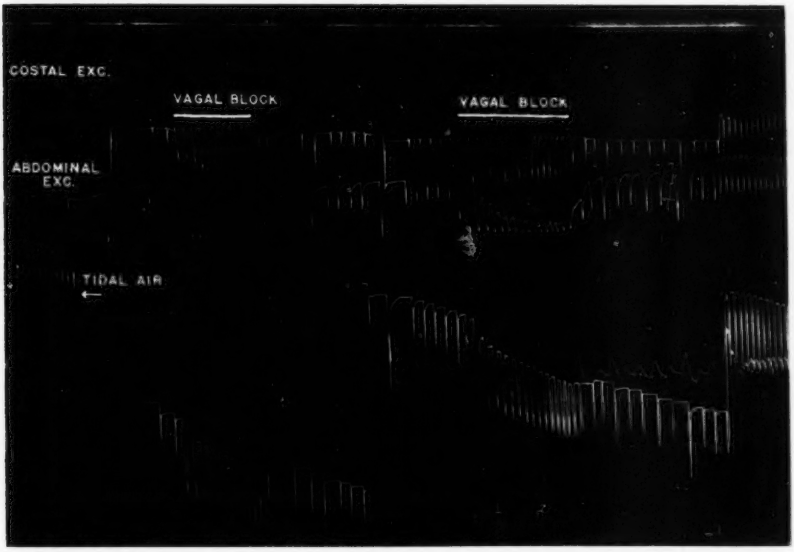

Fig. 1. Effects of exaggeration of the vagal stretch reflex produced by a sustained superinflation of the lungs. For discussion see text.

The simultaneous "inhibition" of the inspiratory act and the increased activation of expiratory muscles considered by themselves are in full agreement with the original Selbsteuerung theory of Hering and Breuer (1868). But the powerful respiratory cycle breaking through the so called inspiratory apnea is most difficult to reconcile with an inspiratory *inhibitory* action of the vagi. The extremely rapid filling of the lungs of this and the next two inspirations to follow cannot be due to the simple mechanical effects of the increased pressure of the air seeking entrance into the lungs. Were that factor important, each expiration should be much *slower* than normal for the air is then leaving the lungs against a greater resistance than normal. On the contrary these expirations are exceedingly rapid. Fur-

thermore, double vagal block during increased intrapulmonary pressure produces striking changes in the respiratory records. Breathing assumes a more swinging gait. Despite a greatly increased depth of breath the velocity of both inspiration and expiration is actually diminished. It therefore seems highly probable that vagal block removes two powerful excitatory effects—one exerted on the inspiratory half center and the other exerted on the expiratory half center. It is well to recall that even Gad (1880), who accepted only an inspiratory inhibitory action of the vagus nerve, noted a greater velocity of inspiratory contractions when the vagus nerves were intact. More recently this same slowing of inspiration after vagotomy has been described by Nicholson and Brezin (1937), and Gesell, Steffensen and Brookhart (1937), and others.

Another striking effect of vagal block during exaggerated reflex vagal activity is the tremendous reduction of expiratory activity in the abdominal segment. The abdomen tends to assume an inspiratory position upon which weakened expiratory contractions are superimposed. This is regarded as conclusive evidence of the powerful excitatory action which the vagi may exert upon the abdominal musculature during excessive inflation of the lungs. Evidence for a similarly increased vagal expiratory activity, however, is missing in the thoracic records for there are no clear indications of constriction of the chest during increased intrapulmonary pressure. As a matter of fact there is a small constriction of the chest during vagal block while the abdominal circumference is increasing. Whether this opposite effect upon the chest is of reflexogenic origin or whether it is due to the increased activity of the diaphragm sucking the chest walls in as the diaphragm descends and pushes the abdomen out is not clear from these experiments and must be decided by action potential studies.

The dual excitatory action of the vagal stretch reflex mentioned above is not without precedent in other forms of respiratory stimulation; e.g., Gesell and White (1938) have shown that the effects of momentary excitation of the chemoceptors of the carotid body vary with the time that such excitation occurs in relation to the respiratory cycle. Thus cyanide injected into the carotid artery, timed to reach the carotid body during the phase of inspiration, will strengthen that particular inspiratory act, while injection timed to stimulate the chemoceptors during the expiratory phase of breathing will intensify that particular expiration. Since there are no apparent reasons for dividing the chemoceptors into inspiratory and expiratory groups there are no apparent objections to assuming that each chemoceptor possesses double central connections, one group of endings synapsing in the inspiratory half center and the other group synapsing in the expiratory half center (see fig. 2). The number of endings in the half centers would thus determine only the relative strength of inspiratory and expiratory stimulating action of the chemoceptors. The same prin-

ciple may be applied to any form of sensory drive. Since stretching of the lungs is found to stimulate both inspiratory and expiratory muscles, as does the discharging carotid body, there is need of assuming but one active set of proprioceptive end organs in the vagal stretch reflex and that such end organs possess dual connections at the center. Since many of these end organs never cease firing (Adrian, 1933) but discharge with a waxing

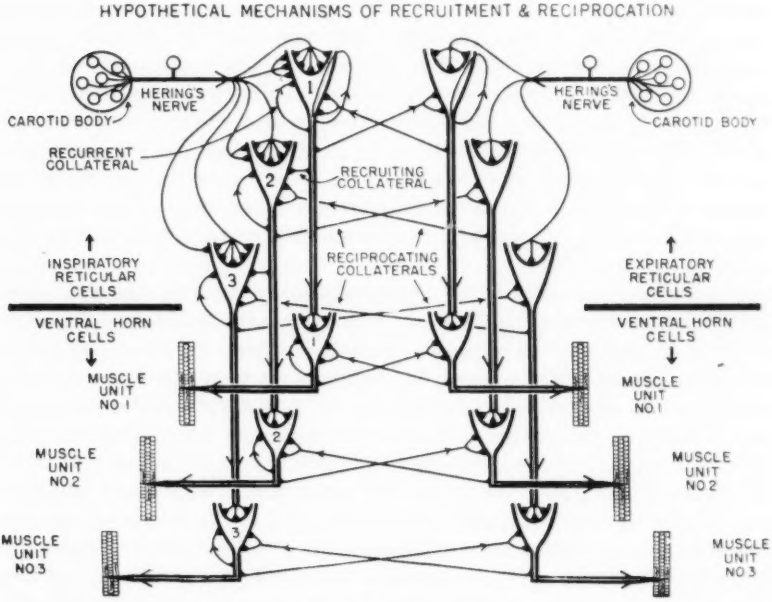

Fig. 2. A schematic representation of the dual connections of the chemoceptors and of the reciprocating connections between half-centers which permit a steady chemoreflex drive to produce a rhythmic alternating inspiratory and expiratory activity. Similar inspiratory and expiratory connections of the individual vagal stretch receptors (see text) should provide a means for shifting the periodic vagal drive along with the steady chemical drive from one half-center to the other with each changing phase of respiratory activity.

frequency during the inspiratory phase and with a waning frequency during the expiratory phase, they are theoretically capable of driving both acts of respiration in a manner similar to the chemoceptors. This we believe is possible through the intervention of reciprocating connections between half centers.

The electrotonic theory of nerve cell discharge offers an extremely simple mechanism of reciprocal inhibition and of alternating activity of half-

centers (see fig. 2). While the inspiratory reticular cells 1, 2 and 3 are discharging they are assumed to dispatch signals via their reciprocating collaterals to the inhibitory poles of the expiratory reticular cells. Theoretically this opposes the prevailing excitatory current in the expiratory cells and holds their discharge in check. Consequently those signals impinging on the inspiratory half center during the normal filling of the lungs fit in with the phase of inspiration and, therefore, strengthen and speed the inspiratory discharge. On the other hand, those signals simultaneously impinging upon the expiratory half center produce only local synaptic action because the negativity they establish is effectively opposed by the reciprocal inhibition exerted by the inspiratory half center. This condition prevails so long as the inspiratory cells discharge, but once their activity ceases, reciprocal inhibition, or local negativity at the inhibitory pole of the expiratory cells is withdrawn. Only then do the excitatory signals of the vagal endings impinging upon the expiratory cells force these cells to discharge. Conversely the expiratory discharges hold the inspiratory center in check.

Since expiratory activity is importantly determined by the number of vagal signals impinging on the expiratory half center during the expiratory phase it is necessary to recognize that the number of these signals diminishes as the lungs collapse. In contrast to inspiratory contraction, expiratory contractions should, therefore, be strong at the outset and weak at the close as they actually are in a sensitive reflex preparation. Accordingly, reciprocal inhibition of the inspiratory center must weaken as expiration progresses. Since expiratory activity, as witnessed by muscular contraction, is relatively weak or even missing under normal conditions there is relatively little or no restraint upon the development of new inspiratory discharges. When, however, the vagal stretch reflex is intensified by a super inflated condition of the lungs, such as seen in figure 1, the resulting expiratory activity becomes a most important regulatory factor. Heightened reciprocal inhibition of the inspiratory half center holds the inspiratory cells in check until the growing chemical stimulation, central and reflex, increases the electrotonic excitation current above threshold.

Granting that the strength of the current leaving the axon hillock determines the degree of nerve cell activity, a sudden withdrawal of negativity at the inhibitory pole should have excitatory action equal to that of added negativity at the excitatory pole. On that basis half centers should not only exert a reciprocal inhibition on each other but also derive a reciprocal excitation from each other. Such dual reciprocating relationships suggest a tangible explanation of the phenomenon of rebound illustrated in figure 1. It will be seen that the sudden and powerful inspiratory contraction interrupting the initial apnea changes most abruptly into an equally sudden and powerful expiratory contraction. The great strength of the expiratory

contraction, we believe, is a rebound effect explained by a *sudden* withdrawal of a powerful inhibition (or negativity at the inhibitory poles) from the expiratory half center at the very end of inspiration. Immediate and sudden withdrawal of negativity is regarded as important in rebound due to the shortness of the local after-synaptic action continuing in the immediate vicinity of the individual synapses. Thus a sudden and deep inspiration is matched with an equally sudden and deep expiration whereas a more shallow but sudden inspiration (see following 2 respirations) is followed by an equally sudden and shallow expiration.

The irregularity of depth of breathing during super vagal activity such as is seen during the period of pulmonary inflation has been a consistent effect and is readily removed by vagal block. This uneveness of depth seems unrelated to a varying frequency of breathing for a deep breath may occur after a short as well as after a longer expiratory pause. We are inclined to attribute the variations to an uneven intensification of two phenomena. The intensification of the inspiratory discharge resulting from the augmented vagal drive should by itself *increase* the depth of breathing whereas the resulting hastening of exhaustion of the inspiratory half-center should cause a premature cessation of the inspiratory discharge and *decrease* the depth of breathing. This is but an extension of the theory of interruption of the normal eupneic inspiration (Gesell, Atkinson and Brown, 1940) in which a rapidly increasing excitation threshold overtakes a more slowly increasing electrotonic excitation current and thus cuts off the discharge. Since elimination of vagal action eliminates an important factor accelerating each individual inspiration it allows the discharge to develop at a slower rate (see fig. 1) and thus reduces the exhaustion factor. As a result the inspiratory discharges reach a greater and more uniform intensity which is reflected in deep and more uniform breathing.

SUMMARY AND CONCLUSIONS

Increased intrapulmonary pressure was found to produce several important respiratory effects throwing light upon the nature of the vagal stretch reflex.

1. The well-known reduced frequency of breathing.
2. A prolonged and increasing constriction of the abdomen.
3. An increased velocity of the inspiration and expiration of air.

Double vagal block during exaggerated vagal stretch reflex activity abolished abdominal constriction and decreased the velocity of both inspiration and expiration while at the same time it increased the depth of breathing.

It is, therefore, suggested that the vagal stretch reflex is an excitatory phenomenon in which each stretch proprioceptive ending drives both the inspiratory and expiratory half centers by virtue of dual connections at

the center. Coördinated alternate reflex activation of the two half centers is dependent upon reciprocating interconnections.

Vagal inhibition of the inspiratory half center is regarded as a secondary response to vagal excitation of the expiratory half center and vice versa. Thus a reduced frequency of breathing produced by an increased volume of the lungs is explained by an increased activity of the expiratory half center exerting a more powerful reciprocal inhibition of the inspiratory half center.

A mechanism is proposed to explain the nature of vagal action and of "rebound."

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ON THE ORIGIN OF THE EXPIRATORY ACTIVITY PATTERNS[1]

ROBERT GESELL AND JOSEPH J. WORZNIAK

From the Department of Physiology, University of Michigan, Ann Arbor

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Three types of respiratory activity patterns are now well established for the dog: 1, the slowly augmenting and rapidly waning; 2, the steady state, and 3, the rapidly augmenting and slowly waning (Gesell and White, 1938; Gesell, Magee and Bricker, 1940; Gesell, Atkinson and Brown, 1940). The first is associated with the inspiratory phase of breathing, the second and the third with the expiratory phase. The slowly augmenting pattern in which activity builds up gradually and subsides abruptly is well adapted to meet the increasing resistance of the lungs and torso during inspiration and of allowing a rapid passive emptying of the lungs during the expiratory period. Passive recoil of the lungs and torso is in addition commonly associated with either a steady state or a rapidly augmenting and slowly waning contraction of the expiratory muscles. The mechanical value of the steady state *contractions* to the movement of air must however be questioned for they so often begin after the lungs have already collapsed to their expiratory volume. On the other hand, their timely *relaxations*, accommodating the following inspiration of air, have definite mechanical value in the inspiratory act. It is, therefore, inadvisable to classify the steady state contraction as purely respiratory for it undoubtedly has a joint function of which the maintenance of visceral posture may be of equal or even greater importance. The rapidly augmenting and slowly waning expiratory contraction is, however, better adjusted to assist the expulsion of air. Its great initial power occurring at the very close of inspiration is conceivably designed to bring pressure on the rapidly receding lungs of passive expiration.

Since elimination of the rhythmic respiratory proprioceptive signals by curari paralysis plus temporary cessation of artificial ventilation does not eliminate the slowly augmenting inspiratory discharges (Gesell, Atkinson and Brown, 1940) this pattern of activity is attributed to the ability of the inspiratory half center to react to a steady form of stimulation with periodic discharges of the slowly augmenting type. In the absence of equally positive evidence on the origin of the expiratory activity patterns

[1] This study was supported by a grant from the Rockefeller Foundation.

a more tentative suggestion was offered in which the steady state discharge was regarded as the basic central pattern of expiratory activity (it, too, was not abolished by curari) and the rapidly augmenting and slowly waning discharge a reflexogenic modification of the steady state discharge. It was pointed out that the action of a powerful but waning discharge of the vagal stretch receptors upon the expiratory half center beginning with the expiratory discharge might explain the origin of the rapidly augmenting and slowly waning expiratory contraction. Our present experiments deal more directly with this problem.

METHODS. Expiratory activity was studied with the aid of muscle action potentials during varying intensities of the vagal stretch reflex, produced by rhythmic artificial inflation of the lungs. The choice of muscle proved to be a most important factor for when the abdominal expiratory muscles were employed results were extremely irregular. It was finally concluded that the uncertainty of response was related to an opposing action of extra vagal reflexes for when the lungs are inflated there is not only a stretching of the receptors in the lungs but in the muscle under study as well. It, therefore, seemed imperative to search for more simple experimental conditions. This finally resulted in the use of the thyroarytenoid muscle which contracts normally during the expiratory period. Since the tracheal cannula is inserted below the larynx, local proprioceptive reflexes produced by inflation of the lungs are avoided and the sensory modifications of expiratory activity become more purely vagal. As a further precaution in the same direction the anterior and lateral chest walls were removed to minimize the effects of proprioceptive signals arising in the thoracic cage. These restrictions, we believe, explain the exceptional uniformity of our results.

RESULTS. The breathing of an artificially ventilated animal, as is well known, tends to fall into phase with the stroke of the pump provided reflex sensitivity is high. For reasons which need not be considered now, either expiration or inspiration may coincide with pulmonary inflation. This dual response proved most helpful in establishing the nature of expiratory activity. In figure 1, for example, expiratory activity, indicated by the electrogram, falls into phase with artificial inflation of the lungs. As the piston descends and the lungs distend, intratracheal pressure rises slowly, and when the expiratory exhaust valve opens and the piston rises the lungs collapse more suddenly and the intratracheal pressure falls abruptly to zero. The correspondence between the frequency of twitch and the rate of rise and fall of respiratory volume, as indicated by the tracheal pressure record, is a most significant point. It recalls the findings of Adrian (1933) on the vagal stretch receptors in which a similar relation of frequency of discharge to lung volume change occurred. Since the response of the thyroarytenoid muscle to lung volume changes disappears

on vagal block it is concluded that the vagal stretch receptors are capable of driving the thyroarytenoid muscle in a precise and machine-like manner. This action is in agreement with views previously proposed of the dual excitatory action of the vagal stretch reflex upon the inspiratory and expiratory half centers (Gesell and Moyer, in press).

It is pertinent that figure 1 was obtained during an apneic condition of the respiratory center produced by excessive pulmonary ventilation (when the pump was stopped the animal failed to breathe), for it emphasizes that vagal activity is capable of eliciting a purely reflex expiratory contraction. While Hering and Breuer were among the first to demonstrate an expiratory action of the vagal stretch reflex they made no distinction

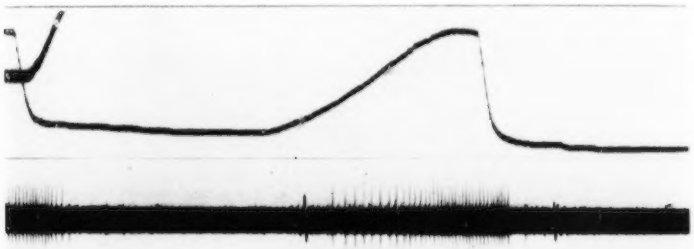

Fig. 1. The electrical response of a single muscle unit of the thyroarytenoid muscle to artificial inflation of the lungs. The gradually rising pressure in the trachea (upper record) indicates a relatively slow and uniform increase of lung volume, while the sudden drop of pressure indicates a more rapid recoil of the lungs. Excepting for the short "after-discharge" following recoil there is a very close relation of frequency of twitch to the prevailing intensity of the vagal stretch reflex.

between a purely reflex and a modified expiratory activity nor were they interested in activity patterns. These points are now considered.

First of all it will help to bear in mind that the increasing vagal activity which occurs during the progressive filling of the lungs in normal inspiration should theoretically produce a progressive intensification of the waxing inherent inspiratory discharge, whereas the decreasing vagal activity occurring during the emptying of the lungs should withdraw stimulation from the expiratory center as expiration progresses and produce a waning expiratory discharge (Gesell, 1940). Such hypothesis agrees with respiratory activity patterns now established. It is, therefore, interesting to note that the expiratory contraction illustrated in figure 1 is contrary to the activity patterns of normal breathing for it is of the slowly augmenting type characteristic of inspiratory activity. But this is readily explained, for we have already shown that the intensity of a purely vagal reflex contraction varies directly with the intensity of discharge of the vagal stretch

receptors. Thus the atypical expiratory pattern is a direct result of the slow augmentation of vagal activity which occurred during the *artificial* inflation of the lungs.

Conversely an artifically created vagal discharge of waning intensity made to impinge upon the expiratory center during the expiratory phase of breathing, should produce a rapidly augmenting and slowly waning pattern of expiratory activity. Such impingement was accomplished in figure 2. Fortunately the frequency of the artificial inflation of the lungs was slow.

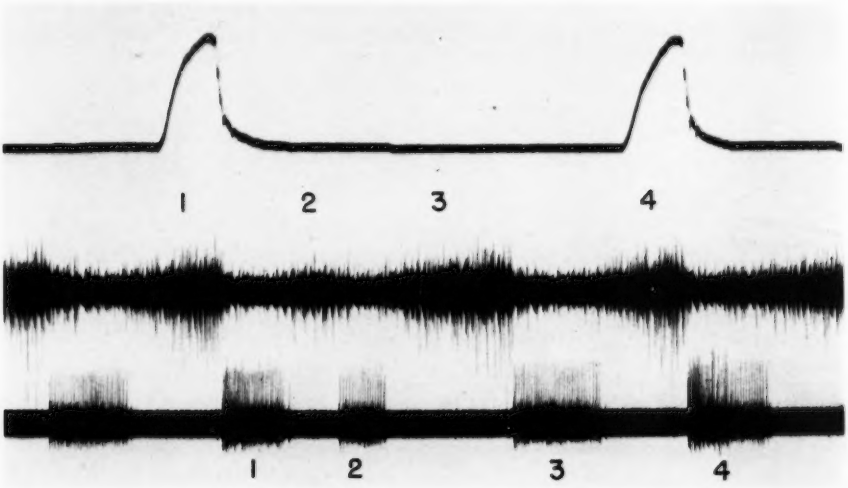

Fig. 2. Effects of intensity of discharge of the vagal stretch receptors on the contraction of the thyroarytenoid muscle. The upper record of tracheal pressure shows artificial inflation of the lungs during open pneumothorax. The upper electrical record shows fusillade contractions of the diaphragm. The lower record shows the activity of the thyroarytenoid muscle. During respiratory cycles 2 and 3 the lungs are collapsed and vagal expiratory drive is uniformly low. During cycles 1 and 4 the vagal expiratory drive is of the slowly waning type. For further discussion see text.

This had the advantage of bringing only every third inspiration (1 and 4 of the upper electrogram) into phase with each artificial inflation of the lungs and allowing two intervening respiratory cycles with the lungs permanently collapsed (2 and 3) to be used for controls. We are thus enabled to compare the activity of the expiratory center under an initially powerful but waning vagal drive (expirations 1 and 4) with the activity under a uniform or zero vagal drive (expirations 2 and 3). It will be seen at once that the discharge is of the steady state type in expirations 2 and 3 and of the rapidly augmenting and slowly waning type in expirations 1 and 4. Since this modification of expiratory contractions by artifical ventilation

of the lungs is usually completely abolished during double vagal block it is concluded that the vagal stretch reflex is probably the most important factor contributing to the slowly waning expiratory contraction during normal breathing. The results of figure 2 combined with figure 1 show furthermore that vagal activity is not only able to elicit a purely reflex contraction but can reflexly modify the steady state activity such as occurs during zero or uniformly small vagal drive. From this it follows that the rapidly augmenting and slowly waning expiratory contraction can be either a purely reflex activity or a modification of the steady state activity.

What then is the nature of the expiratory discharges and wherein do they differ from the slowly augmenting inspiratory discharge? Acceleration of frequency in the latter discharge (since it was obtained in the absence of all periodic afferent impulses) was tentatively attributed to the augmenting action of the recurrent collaterals (Gesell, Atkinson and Brown, 1940). Since acceleration is missing in the steady state discharge these structures have been omitted in the architectural schema of the expiratory half-center. Recruitment of new active units in the course of a slowly augmenting discharge has also been explained on a structural basis, but with the aid of recruiting collaterals. Since recruitment is absent in the course of a steady state contraction the recruiting collaterals have been omitted in the expiratory half center schema as well. While it is true that the steady state activity may vary with varying intensity of chemical stimulation, it still retains the steady state pattern. Such increased activity is regarded simply as an activation of a greater number of cells within the subliminal range. The relatively greater number of expiratory units active at the beginning of a rapidly augmenting and slowly waning contraction is accordingly attributed to an initially intense reflex activation of those cells lying in the subliminal fringe. This activation however diminishes as contraction progresses and vagal discharge weakens, thus producing the familiar phenomenon of decruitment.

The relatively greater incidence of the steady state expiratory contraction as compared with the rapidly augmenting and slowly waning contraction in routine electrical exploration of expiratory muscles is possibly related to the low degree of reflex sensitivity prevailing under ordinary experimental conditions. On the other hand it may be an indication of the relative unimportance of expiratory activity in the mechanics of eupneic breathing.

SUMMARY

The nature and origin of the expiratory activity patterns were investigated by recording the expiratory action potentials of the thyroarytenoid muscle of the dog during changing vagal activity controlled by artificial inflation of the lungs.

Intensity of contraction was found to vary with the degree of pulmonary

inflation. Since this effect was abolished by double vagal block it was concluded that the vagal stretch reflex is capable of driving expiratory muscles in a precise and machine-like manner.

When the lungs were inflated in a progressive manner the synchronous expiratory contraction was therefore of the slowly augmenting type. When however an initially high but slowly waning vagal discharge occurred in phase with the expiratory discharge a rapidly augmenting and slowly waning contraction was obtained. It was therefore concluded that the waning vagal activity occurring during normal expiration is an extremely important factor in the production of the rapidly augmenting and slowly waning expiratory contraction.

Architectural arrangements of the inspiratory and expiratory half-centers underlying the slowly augmenting and steady state contractions are discussed.

Since vagal activity was demonstrated to evoke a purely reflex activity as well as a reflex modification of a prevailing expiratory discharge it is concluded that the rapidly augmenting and slowly waning expiratory activity may be primarily a reflex response or a reflex modification of the steady state discharge.

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INFLUENCE OF RIGHT AND LEFT VENTRICLES ON THE ELECTROCARDIOGRAM[1]

H. E. HOFF, L. H. NAHUM AND BRUNO KISCH[2]

From the Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn.

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To explain the genesis of the normal electrocardiogram, earlier writers have postulated the summation of electrical events from the two ventricles, each ventricle acting as a unit, and giving rise to its own electrogram. These are designated as dextro- and levocardiograms, and are believed to be of opposite polarity, and slightly out of phase with one another (1). By blocking first one and then the other bundle-branch, following suggestions of Eppinger and Rothberger (2), Lewis obtained what he considered to be the initial portion of the dextro- and levocardiograms, and showed that their algebraic summation resulted in a curve that resembled the initial deflection of the normal electrocardiogram taken before the experimental procedures (3). Since this method did not extinguish activity in one or the other ventricle, but only postponed its occurrence, it was impossible to determine the nature of the entire dextrocardiogram or levocardiogram. Despite continued interest in the electrograms of the right and left ventricles (4), efforts to obtain complete curves, and to determine if they would summate to yield a normal T wave have hitherto been unsuccessful. There is, however, evidence that the character of the T wave in electrograms taken by direct leads "is determined by the algebraic summation of the monophasic action current recorded by each of the electrodes" (1, 5).

In these experiments we have obtained electrograms from the right and left ventricles by a method which reduces or extinguishes electrical activity at the surface of the opposite ventricle. This method has been described previously (6, 7) and is based upon the following two observations: *a*. The electrocardiogram is determined by activity at the surface of the heart. *b*. Potassium chloride in M/10 or M/5 solutions extinguishes electrical activity at the point of its application to the surface of the heart. By covering large portions of the surface of a single ventricle with filter paper

[1] This work was aided by a grant from the Fluid Research Funds, Yale University School of Medicine.

[2] Fellow, Emergency Committee in Aid of Displaced Foreign Scientists.

soaked in M/5 KCl solution, it was thus possible to expose the electrogram of the untreated ventricle.

METHOD. Seven dogs, 12 cats, and 6 monkeys (5 rhesus and 1 mangabey) were employed in these studies. Animals were deeply anesthetized with sodium amytal and the heart exposed as described previously (6). Care was taken to preserve as much as possible of the over-lying soft tissue, and this was clipped together during recording to restore normal continuity as far as possible and to permit adequate conduction from this area. For the same reason the lungs were fully inflated during recording. A small slit only was made in the pericardium, so that this structure remained intact and served to hold the KCl pledget in place. Electrocardiograms were taken from the three conventional leads with the animal placed on its side, since it was found that the most striking changes were obtained with the animal in this position (8). Fifth molar potassium chloride solution was employed throughout these experiments.

The major difficulty encountered was caused by spread of solution from the area being studied. To avoid this it was necessary to remove all excess fluid from the pericardium, and from the chest cavity after each washing, and to use relatively dry pledgets. With these precautions spread of solution was minimized, and it was also found that the pledget itself showed less tendency to change its position. After each treatment, the pledget was removed, the heart and pericardium were thoroughly washed with warm Ringer-Locke solution, and time was allowed to permit the electrocardiogram to return to normal (6).

RESULTS. When a large portion of the surface of a single ventricle was treated, an electrocardiogram was obtained which resembled closely the monophasic records that have been derived directly from the surface of the heart (5, 8, 9). The record obtained by extensive damage to the left ventricle was upright, and began with a short downward Q wave, and its rapid upward limb was the R wave. Since it was produced by damage to the left ventricle, and since it preceded the opposite wave, it was interpreted as the record of preponderant electrical activity in the right ventricle, or the dextrocardiogram (fig. 1B). The monophasic-like record obtained by treatment of most of the right ventricle with potassium chloride was directed downward, and its rapid descending limb was the S wave. It was interpreted as a record of the electrical activity of the left ventricle, or the levocardiogram (fig. 1C).

When these curves were plotted using the Q wave as a point of reference, and the major deflections summed algebraically, a complex was obtained which closely resembled the normal electrocardiogram (fig. 2). In experiments in which an inverted T was present it was observed that the durations of the dextro- and levocardiograms, measured between the major deflections at the isoelectric level, were equal. Consequently the levocardiogram persisted for some time after the termination of the dextrocardiogram, since it commenced somewhat later. In a single experiment in which an upright T wave was observed, the levocardiogram was of shorter duration than the dextrocardiogram, so that even though it began later, it was completed

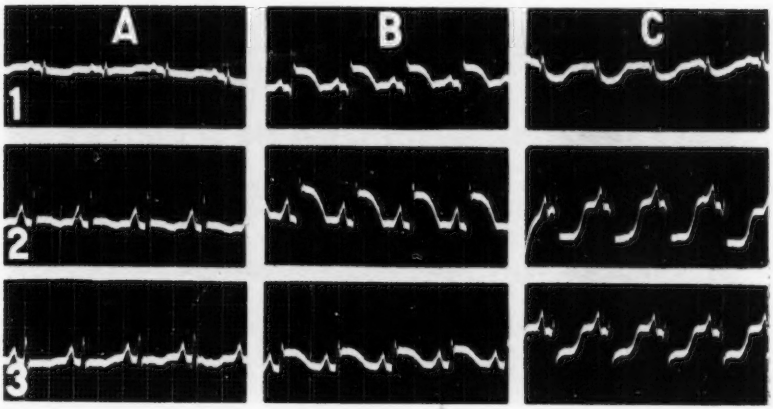

Fig. 1. Dog. November 9. A. Control, leads 1, 2 and 3. B. Dextrocardiograms in the three leads. C. Levocardiograms in the three leads. The levocardiogram is imperfect in lead I.

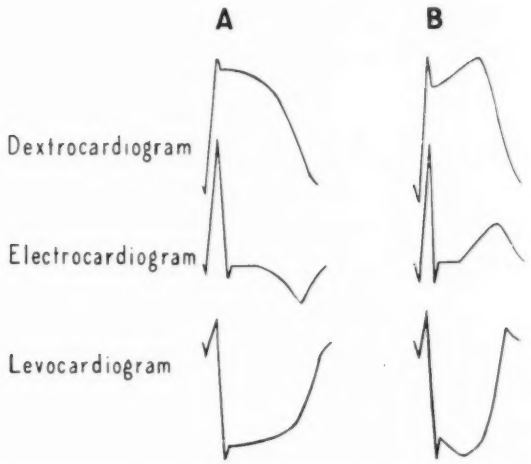

Fig. 2. A diagram illustrating the factors regulating the direction of the T wave. In A the dextro- and levocardiogram are of equal duration, but the levocardiogram, starting later, ends later. A negative T wave would result as shown in the center figure, which represents the actual summation. In B the levocardiogram begins later, but is of shorter duration, so that it ends earlier than the dextrocardiogram. This would form an upright T wave.

before the termination of the dextrocardiogram (fig. 3). This relative shortness of the levocardiogram was accentuated when the animal was cooled (fig. 3).

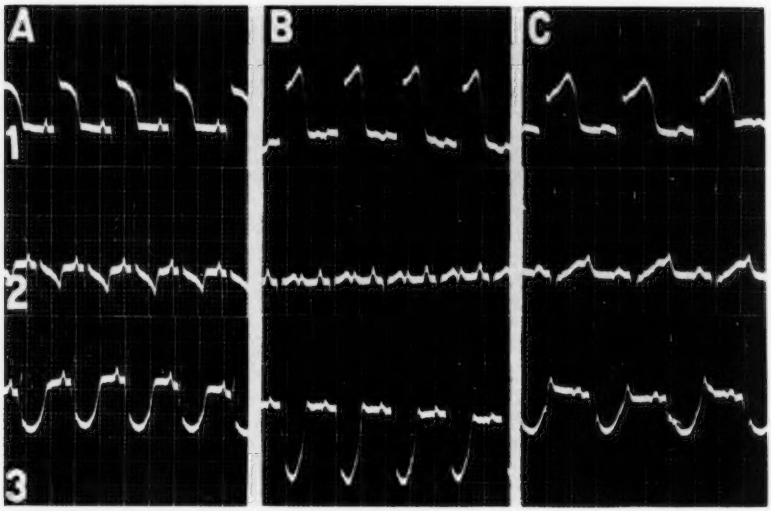

Fig. 3. In A are shown the above dextrocardiogram and below the levocardiograms from lead II of a typical experiment in which the T wave was inverted. The duration of the two electrograms is the same. In B are shown the dextro- and levocardiograms from an experiment with an upright T. The duration of the levocardiogram is such that despite the later onset, it ends 0.02 to 0.03 sec. before termination of dextrocardiogram. There is also a distinct difference in contour. Both factors are accentuated in C, taken with the animal cooled to 30°C. Number 2 in each group shows the control electrocardiogram, also from lead II.

Discussion. In the experiments reported here we have succeeded in recording what appear to be the electrograms of individual ventricles. This was accomplished by extinguishing the electrical activity of one ventricle by covering its surface with M/5 potassium chloride, which permitted registration of the contribution made to the electrocardiogram by the opposite ventricle. The effects of potassium chloride were rapidly reversible, permitting the recording of both the dextro- and the levocardiogram from the same heart.

A striking similarity was found between the electrogram of an entire ventricle derived from the conventional leads and the monophasic action current obtained from a single point on the surface of the heart. There were, however, some variations between the dextro- and levocardiograms and pure monophasic curves. The dextrocardiogram always showed a Q

wave whenever this was present in the electrocardiogram. The levocardiogram showed a Q wave and a small R wave. Inasmuch as technical reasons make it impossible completely to cover the ventricle with the solution, it is possible that the R wave in the levocardiogram was a remnant of right ventricular activity not abolished by potassium chloride. The more complete the coverage, however, the less prominent the R wave became.

The dextro- and levocardiograms usually showed a sharp spike before the plateau of the wave. This spike is frequently seen also in monophasic action currents. It tended to be reduced as the block of the ventricle by potassium chloride became more complete, and may therefore not represent a characteristic of the curve from the opposite ventricle. It was, however, seen so frequently that it cannot yet be ruled out as a part of the complex.

In all conventional leads the dextrocardiogram was upright, and the levocardiogram was inverted. The dextrocardiogram preceded the levocardiogram by a short interval, and its ascending limb was the R wave. The apex of the dextrocardiogram thus coincided with R, and the apex of the levocardiogram with S. In the dog the levocardiogram therefore began approximately 20 msec. after the onset of the dextrocardiogram.

Some conclusions may now be drawn concerning the genesis of the electrocardiogram. The monophasic character of the electrogram of each ventricle indicates that each ventricle fires as a unit with practically simultaneous discharge of all its fibres. The right ventricle is activated first, and contributes Q and the upstroke of R. At this time the left ventricle is activated, and the potential is equalized, forming the downstroke of R and S. During the ensuing S-T interval both ventricles are active, but their opposite electrical potentials neutralize each other at the distant leads. If cessation of activity in the right ventricle occurs before that of the left, a downward T wave results. This is the usual finding in the dog in these experiments (figs. 2 and 3). Occasionally, the dextrocardiogram persists after the levocardiogram has ended, even though it began earlier. In such cases, the T wave is upright, because there is now a residue of the upright dextrocardiogram (figs. 2 and 3).

The presence of a Q wave preceding both the dextro- and levocardiogram suggests that it may arise in tissue activated before the surface of the two ventricles.

SUMMARY

1. Extinction of electrical activity of the left ventricle by covering its surface with M/5 KCl permits the recording of the electrogram of the right ventricle, which may be designated as the dextrocardiogram. It is a monophasic-like curve which arises from the R wave, and is upright in the three conventional leads.

2. The levocardiogram is similarly obtained by blocking the electrogram of the right ventricle. It is a monophasic-like wave which arises with the S wave, and thus is somewhat later in time than the dextrocardiogram. In all conventional leads it is inverted.

3. The electrocardiogram represents the algebraic sum of the dextro- and levocardiograms.

4. Similar results were obtained from dogs, cats and monkeys.

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THE SIGNIFICANCE OF DISPLACEMENT OF THE RS-T SEGMENT[1]

L. H. NAHUM, H. E. HOFF AND BRUNO KISCH

From the Laboratory of Physiology, Yale University School of Medicine, New Haven, Connecticut

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Certain specific electrocardiographic patterns have come to be associated with the acute and later phases of coronary occlusion (1). In the early phases these consist of elevations or depressions of the RS-T segment, often with elevation in one lead and depression in another. Elevation in lead I and depression in lead III are considered to be due to infarction of the anterior portion of the left ventricle, while depression in lead I and elevation in lead III are interpreted as results of infarction of the posterior wall of the left ventricle (1). The significance of such patterns has been derived chiefly from clinico-pathological correlations, and the reason for these changes remains in doubt.

Experiments reported in a preceding paper indicate that the normal electrocardiogram results from interference between electrograms of the two ventricles, that of the right ventricle preceding by a short interval the electrogram from the left ventricle (2). These electrograms resembled greatly monophasic action currents recorded from the mammalian heart, and in the three conventional leads the dextrocardiogram was upright, while the levocardiogram was inverted.

These electrograms were produced by reducing or abolishing the electrical activity of a single ventricle by covering a large part of its surface with pledgets of filter paper soaked in M/5 KCl. When smaller squares of filter paper were employed less pronounced results were obtained, but they always consisted of elevations of the R-T interval indicating left ventricular damage or depression of the S-T segment indicating damage to the right ventricle. This paper reports experiments which throw further light on the significance of deviations of the RS-T interval, particularly when elevation is found in one lead and depression in another.

METHODS. The experiments were carried out on the animals employed in the previous series (2), using the same method of application of squares of filter paper

[1] This work was aided by a grant from the Fluid Research Funds, Yale University School of Medicine.

soaked in M/5 KCl, except that the pledgets were small enough to cover only a small portion of the surface of the heart. In some experiments the area treated was confined to a single ventricle, while in others contiguous areas of both ventricles were treated. Records were taken with the animal in various positions to determine the influence of this factor on the magnitude of changes.

RESULTS. A. *Single ventricular effects.* When the pledget was placed only upon the left ventricle the alterations observed consisted always of an upward displacement of the RS-T segment, while damage to the right ventricular surface showed invariably a downward displacement (figs. 1–2). The direction of these changes was the same whatever portion of the left or right ventricle was studied, but the magnitude depended upon 1, the size of the area affected; 2, its position on the heart, and 3, the position of the animal. Changes so produced were always maximal in lead II.

The first factor has been emphasized in a previous communication (3), namely, that the extent of the displacement is proportional to the area affected. The other two points may now be reported more fully. The lateral and anterior surfaces of each ventricle were relatively inactive with the animal on its back (fig. 2, D), while the apex and posterior surfaces yielded maximal changes. When the animal was placed on its right or left side, however, treatment of the anterior and lateral surfaces produced changes equalling those from the apex or posterior surfaces (fig. 2, E, F). In monkeys in which the left lung was collapsed, while respiration was maintained by the unaffected right lung, minimal changes only were obtained from the anterior surface of the left ventricle. When the lung was inflated, more striking changes were recorded. Changing the position of the animal from the back to the sides occasionally reduced the extent of RS-T displacement when the pledgets were placed on the posterior surface of the heart. Great care must be taken to insure that the potassium chloride solution does not spread beyond the area treated. This readily happens if excess fluid collects in the pericardial cavity and the position of the animal is altered. In this event both ventricles may be involved and the results will be complicated.

B. *Combined right and left ventricular effects.* When the pledget was placed over the septum so that it covered parts of both ventricles, mixed effects were obtained. Placed anteriorly, it produced in lead I an upward displacement of the RS-T segment, and a downward one in lead III (fig. 3). The downward displacement in lead III was usually greater than the upward deviation in lead I, and as a result, the S-T segment in lead II was usually also depressed.

Combined effects from the apex and whole posterior septal region showed the opposite picture; the RS-T segment in lead I was invariably deflected downward, while in III it was upward. In lead II it was usually elevated (fig. 4). This seemed to be determined largely by the relative areas involved on each ventricle (fig. 4).

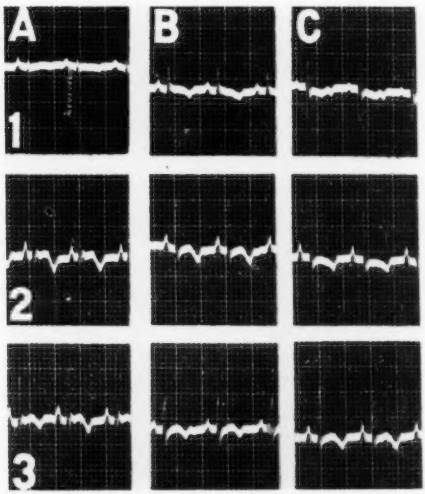

Fig. 1. Dog. November 24: 4.3 kgm. Control in leads I, II, III, with animal on back (A), on left side (B), and on right side (C). These serve as control for figure 2.

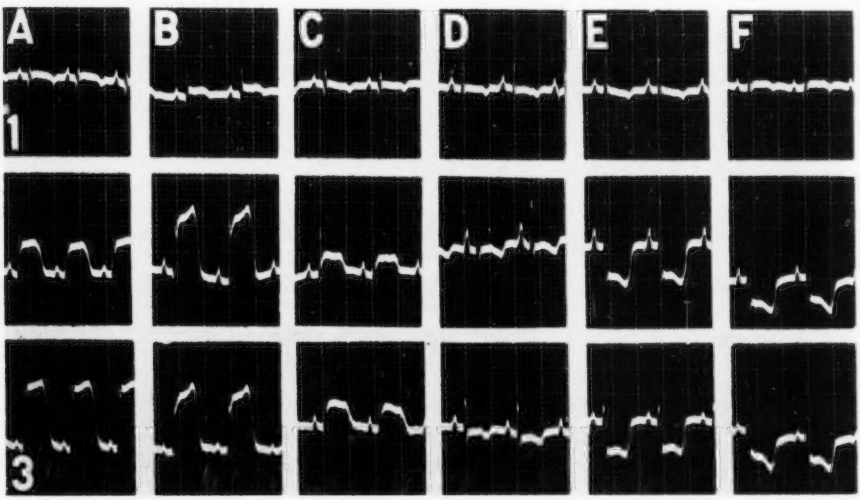

Fig. 2. Same animal as in figure 1. In A, B and C, pledget 2.5 x 1.5 cm. on anterior-lateral surface of left ventricle. Leads I, II, III. Back (A), left (B), right (C). In D, E, and F, the pledget was on the anterior lateral surface of the right ventricle. Animal on back (D), left side (E), right (F). This figure illustrates the elevation produced by purely left ventricular lesions and the depression following purely right ventricular lesions. These changes were most marked when the animal was on its left side, and are most prominent in lead II.

Shift in the position of the animal in these experiments produced marked changes in the magnitude of the effects evoked from the anterior surface (fig. 3, D, E, F), while alterations of position did not influence results from the posterior surface as greatly. Change in the position of the animal did not influence the direction of the displacement, but only its extent, provided that spread of solution was avoided.

In the monkey the left ventricle seemed to be preponderant at the apex and posterior surface, so that frequently a pledget placed at the

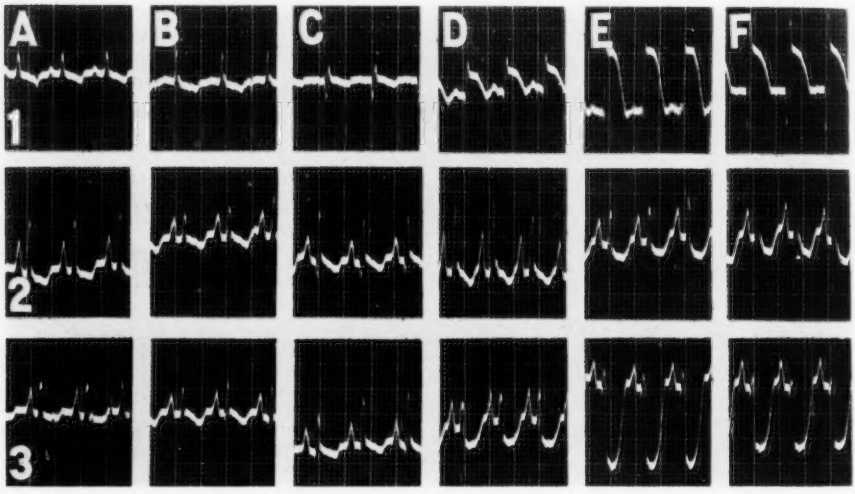

Fig. 3. Dog. January 5; 7.2 kgm. A, B, C, control from back (A), left (B), and right side (C) in leads I, II, and III. Pledget 3.0 x 3.0 cm. over anterior septum at base. Back (D), left (E), right (F). This figure illustrates strikingly the elevation of R-T in lead I and the depression of S-T in lead III produced by an anterior lesion involving adjacent surfaces of both ventricles. Maximal changes were recorded with the animal on its left side. The interference of the dextrocardiogram in lead I and the levocardiogram in lead III produced an almost normal T wave in lead II.

septum produced only left ventricular effects. This may be accounted for by the fact that in the monkey the bulk of the posterior surface of the heart is made up of the left ventricle. In the dog the right ventricle has a larger representation in the posterior surface, and in this animal mixed effects were always obtained.

C. *Changes in Q R S.* Upward deviation of the R-T segment produced by injury of the left ventricle invariably occurred somewhere on the downstroke between R and S. With larger elevations the amplitude of R increased, forming a wave having close resemblance to a monophasic

action current (fig. 2, A, B). When depressed S-T intervals were obtained from right ventricular damage, the take-off occurred somewhat later, after the S wave was completed. In these records, the R wave diminished as the S wave increased until in some records monophasic-like waves were found which were opposite to those obtained from the left ventricle, and occurred a little later in the cycle (fig. 2, E).

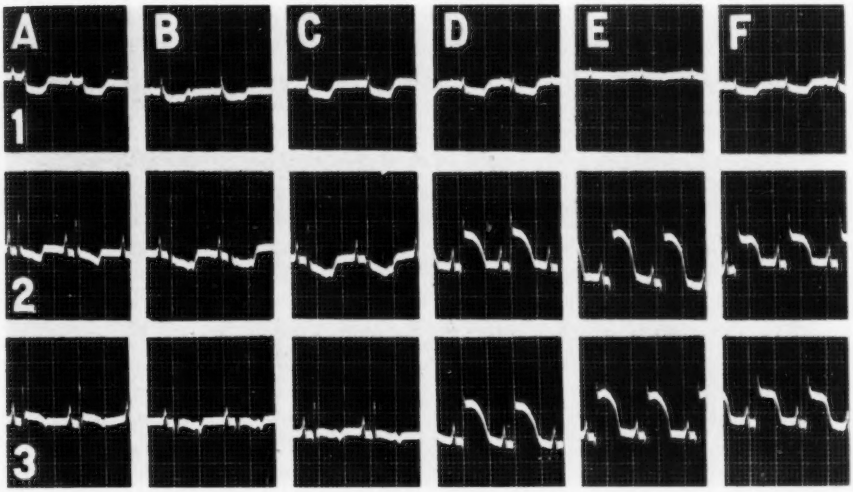

Fig. 4. Dog. November 2; 5.0 kgm. Pledget 1.5 x 1.5 cm. Pledget placed across septum on posterior surface. In A, B, and C it was near the base, and almost entirely on the right ventricle, while in D, E, and F it was nearer the apex and largely on the left ventricle. This figure shows how the magnitude of changes in the various leads is influenced by the relative areas involved. In A, B, and C the pledget was largely on the right ventricle; and both leads I and II show the depression of the S-T segment characteristic of a right ventricular lesion. The elevation of R-T indicative of left ventricular involvement is seen only in lead III when the animal was on its back (A 3). In D, E, and F the pledget was mostly over the left ventricle, and now marked elevation is seen in leads II and III.

Discussion. A. *Significance of RS-T displacement.* These experiments give a definite significance to displacements of the RS-T interval. Elevation of R-T in any lead denotes damage to the surface of the left ventricle, while depression of S-T indicates damage to the right ventricle. The mechanism of these deviations is indicated by the nature of the action of potassium chloride (4). When electrical activity is extinguished in a portion of the heart by potassium chloride, an imbalance is created between the dextro- and levocardiograms, and the undamaged ventricle preponderates, producing either an elevation or a depression of the RS-T segment,

depending upon which ventricle is injured. As more and more of the ventricle is damaged, the opposite ventricle becomes more and more preponderant, until finally pure dextro- or levocardiograms remain (2).

B. *Localization of injury.* When only one ventricle was involved the displacement was similar in all leads regardless of the region of that ventricle affected, and was always maximal in lead II. It was possible to determine whether these lesions were anterior or posterior by the influence of alteration in position of the animal on the magnitude of changes recorded. Electrocardiographic evidence of anterior lesions in either ventricle was always magnified by placing the animal on its side. On the other hand, when the lesion was on the posterior surface of either ventricle, maximal changes were observed with the animal on its back.

More exact localization was possible when adjacent areas of both ventricles were involved. Then lead I became more active than was seen following lesions of single ventricles, and was always opposite to lead III, while lead II showed the least changes. Elevation of RS-T in lead I and depression in lead III characterized anterior lesions, while depression in lead I and elevation in lead III denoted posterior lesions. Lesions at the apex produced changes similar to those of posterior lesions.

The observation that a pledget over the septum involving contiguous areas of both ventricles showed right ventricular effects in one lead and left ventricular in another, indicates that action currents from contiguous areas are not algebraically summated in leads I and III. This will be considered in a subsequent communication.

The electrocardiographic changes produced by pledgets covering part of both ventricles compare closely with those ascribed to acute infarction. Barnes describes the characteristic changes in the standard leads following anterior lesions as an elevation of the R-T segment in lead I and a depression of the S-T segment in lead III (1). When these effects are marked, they have the appearance of monophasic waves. Posterior lesions evoke downward deviation of the S-T segment in lead I, and upward deviation in lead III.

In the experiments reported here, such patterns have been found only as the result of injury to contiguous areas of both right and left ventricles.

C. *Influence of position of animal.* We interpret the influence of change in position as due to alteration in conductivity from the involved areas. The reason that the apex and posterior surface consistently yielded maximal changes with the animal on its back is probably due to the contact of these regions with good conductors, as Lindner and Katz have pointed out (5). Anteriorly, there is little contact with surrounding tissues when the animal is on its back, but when the animal lies on its side, the anterior surface of the heart falls against the root of the lungs and the anterior chest wall, and thus increases its contact with conducting tissues. In this

position, there is a great increase in the effect of anterior applications. This emphasizes further the necessity for adequate conduction from the surface of the heart to reveal the effects of damage, and suggests the use of shift of position as a means of localizing the site of injury. This procedure is the only means of distinguishing anterior from posterior lesions when damage is restricted to a single ventricle, since in these circumstances all leads record either elevation or depression, depending on the ventricle involved.

SUMMARY

1. Elevation of the R-T segment of the electrocardiogram in the dog, cat, and monkey indicates injury to the left ventricle.

2. Depression of S-T indicates injury to the right ventricle.

3. When the injury is restricted to a single ventricle, the RS-T interval in all three conventional leads is deflected in the same direction.

4. Elevation of R-T in one lead, and depression of S-T in another indicate that the damage involves contiguous areas of both ventricles.

5. Elevation in lead I and depression in lead III indicate an anterior lesion.

6. Depression in lead I and elevation in lead III indicate an apical or posterior lesion.

7. Lesions of the anterior surface of the heart are recorded best with the animal on its side, while lesions of the posterior surface of the heart are recorded best with the animal on its back.

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THE FACTORS DETERMINING THE DIRECTION OF THE T WAVE: THE EFFECT OF HEAT AND COLD UPON THE DEXTRO- AND LEVOCARDIOGRAM[1]

H. E. HOFF AND L. H. NAHUM

From the Laboratory of Physiology, Yale University School of Medicine, New Haven, Connecticut

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A satisfactory explanation of the formation of the T wave of the mammalian electrocardiogram is still required. Much work on the subject of the T wave was done by earlier investigators of the electrophysiology of the heart but their studies were carried out on the amphibian heart, and have never been adequately extended to the mammal. In 1880 Burdon-Sanderson and Page (1) first proposed the interference theory to explain the terminal portion of the electrocardiogram of the tortoise. They studied the electrogram obtained by direct leads at the base and apex, and concluded that the initial deflection or "spike" was formed of two elements. The rising phase was considered due to the onset of electronegativity at the base, and its rapid descending phase was supposed to result from the activation at the second electrode at the apex which equalized the potential between the two electrodes, although both regions remained negative to non-active regions. The following isopotential phase they explained as resulting from the interference of the electrical activity at both points. The electrical activity of the heart then ceased in the order in which it began, and therefore negativity at the base subsided while that at the apex continued, producing an inverted end-deflection, or T wave.

The hypothesis was therefore first applied to the interference between apex and base. It was later altered, to apply to interference between the right and left ventricles. Lewis compressed alternately the right and left bundle branches in an attempt to obtain at least the initial portion of the electrogram from each ventricle. Algebraical summation of the initial portions of these complexes did in fact yield a complex comparable to the normal QRS (2).

Concerning the final portion of the electrocardiogram, Lewis wrote: "T can be regarded also as the product of the end-deflections of right and

[1] This work was aided by a grant from the Fluid Research Fund, Yale University School of Medicine.

left ventricle. Unhappily, we have no certain means of ascertaining either the direction or value of the end-deflections of the true dextrocardiogram and levogram. I have in mind the real end-deflections and not those which appear when the left and right bundle branches are divided; for these, as I have explained, are not reliable indications. Experiments in which one or the other ventricle is removed and the resultant curve observed are perhaps too crude to possess material value from this point of view."

In a former communication there has been described a method for obtaining the dextro- and levocardiograms (3). It consisted of temporarily reducing or extinguishing the electrical activity of a single ventricle by the application to its surface of M/5 KCl, so that the unopposed activity of the other ventricle was recorded.

It was seen that complexes thus recorded resembled the monophasic action currents obtained from the surface of the mammalian ventricle, and in all conventional leads, the dextrocardiogram was directed upward, while the levocardiogram was directed downward (fig. 4B). The dextrocardiogram began earlier in the cardiac cycle as a continuation of the R wave, while the levocardiogram was a continuation of the S wave (fig. 4F).

When the duration of each component was the same, the levocardiogram, beginning later, ended later than the dextrocardiogram. Algebraic summation of such complexes would be expected to produce an inverted T wave, and in fact an inverted T wave was found in the normal electrocardiogram. This is illustrated in figure 1A.

In the following experiments further studies of the factors determining the direction of the T wave have been carried out. If the hypothesis be true that the T wave is created by the interference of dextro- and levocardiograms, then alterations in the duration of each component should change materially the direction and contour of the T wave. If the duration of the dextrocardiogram or upward component were shortened, the T wave should become more negative due to the increased amount of unopposed levocardiogram. The duration of the Q-T interval should not be altered. This situation is illustrated in figure 1B. If on the other hand the dextrocardiogram were lengthened, there should then be a residue of unopposed dextrocardiogram, and a positive T wave should occur, as in figure 1C. Here the duration of Q-T should be prolonged. The same considerations apply to changes in the levocardiogram, with the dextrocardiogram remaining normal. Here opposite effects would be expected as illustrated in figure 1D and E. Shortening the levocardiogram should produce an upward T, without changing the duration of the complex, while lengthening the levocardiogram should result in an inverted T of longer duration.

It is known that heating and cooling the animal as a whole shortens and

lengthens the ventricular complex of the electrocardiogram. In these experiments heat and cold were applied separately to each ventricle in an attempt to shorten or lengthen single components of the electrocardiogram, and to determine whether the results thus obtained would confirm the theoretical predictions made above.

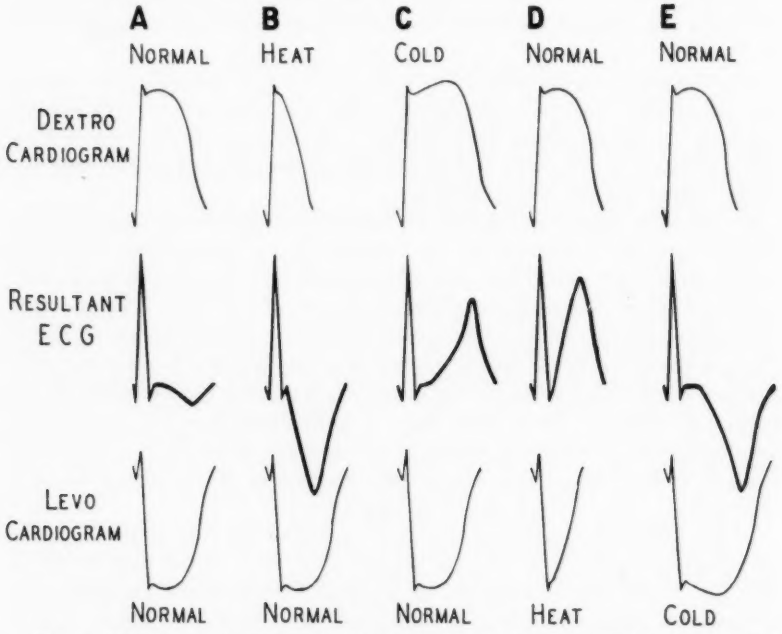

Fig. 1. This figure illustrates the theoretical basis for the experiments reported here. In A are shown at the top a normal dextrocardiogram, and below a normal levocardiogram. Their relative proportions are taken from previous experiments. The dextrocardiogram shows a Q, while both a Q and a small R are shown in the levocardiogram. In the middle is shown the result of an algebraical summation of the dextro- and levocardiograms. Since the duration of the two components is the same, measured from the major deflection at the isoelectric line, the dextrocardiogram terminates before the levocardiogram, and a negative T results. In B and C are shown the results on the T wave of shortening and lengthening the dextrocardiogram, while the levocardiogram remains normal. In D and E the dextrocardiogram remains normal while the levocardiogram is shortened and lengthened. When either the dextro- or levocardiogram is prolonged, a prolonged Q-T interval results.

METHODS. Seven dogs were employed. They were anesthetized with sodium amytal, and the heart exposed under artificial respiration, through an incision over the fifth left rib. Electrocardiograms were obtained from the three conventional leads with the animal on its right side, with the lungs fully inflated and the skin wound closed with clips.

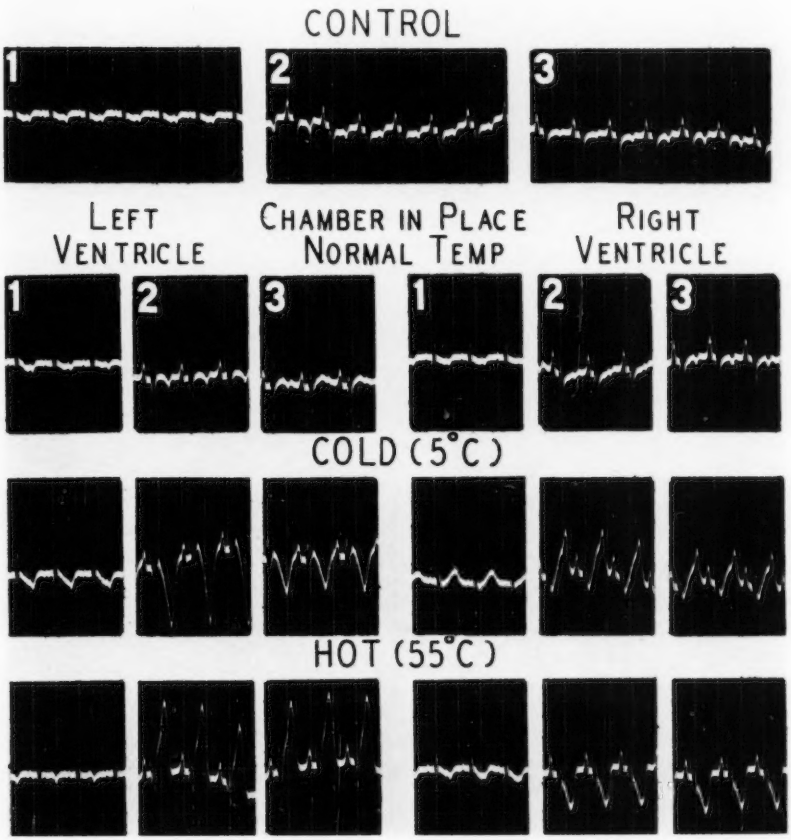

Fig. 2. April 29, 1940; 9.5 kgm. male dog. At the top are control records from leads 1, 2 and 3, taken after all operative procedures. All records were taken with the animal on its right side, with the lungs fully inflated, and the skin wound closed by clips. Next below are controls with the chamber in place on each ventricle, but at body temperature (35°-36°C). Cooling the left ventricle produces a negative T while cooling the right ventricle produces an upright T. As can be seen readily from the relation to the P wave, these complexes are longer than normal. Finally are shown the effects of heating the left ventricle and the right ventricle. Here it is seen that the whole complex is of normal duration.

Areas of each ventricle were cooled or warmed by means of a thin hollow chamber of pure tin curved to fit the surface of the heart. Through this chamber was circulated water from reservoirs at about 55°C. and 5°C. The diameter of the disk was 3.0 cm., and its thickness 3.0 mm. Dextro- and levocardiograms were obtained by applying to the surface of the heart squares of filter paper soaked in M/5 KCl.

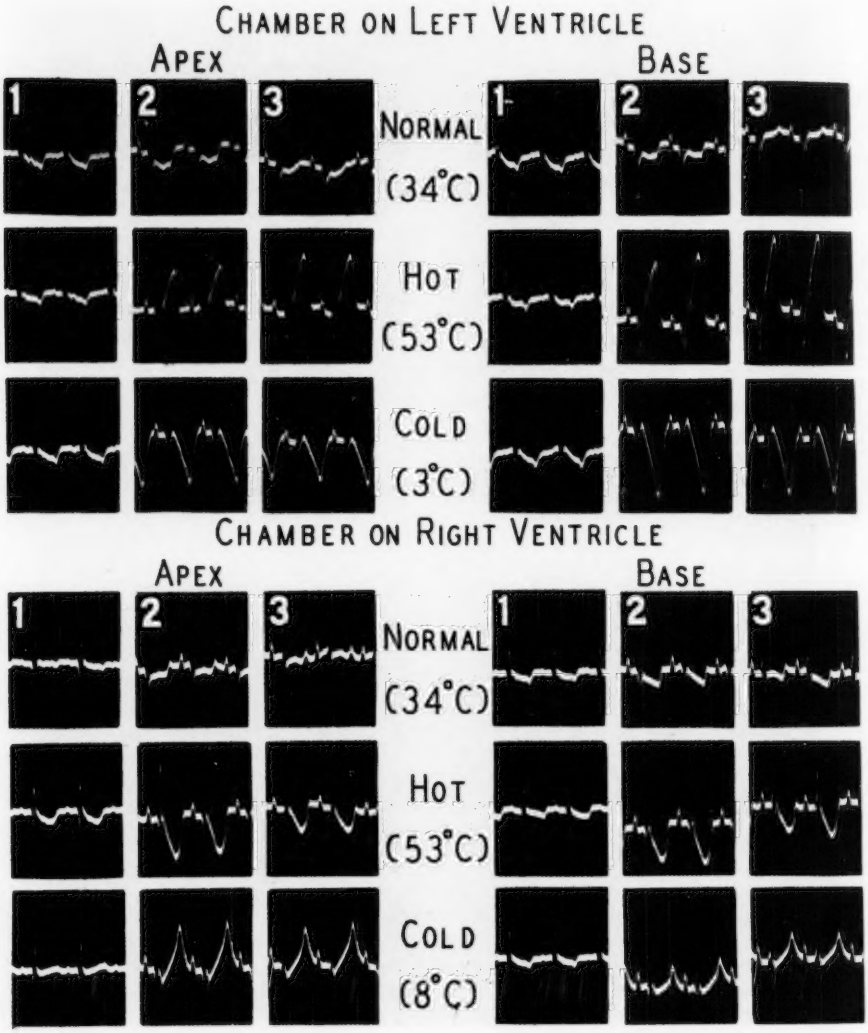

Fig. 3. June 14, 1940: 10.0 kgm. male dog. The effect of heating and cooling the base and the apex of each ventricle is shown. The character of the alterations in the T wave is the same. The only differences are slight variations in the magnitude of the effects.

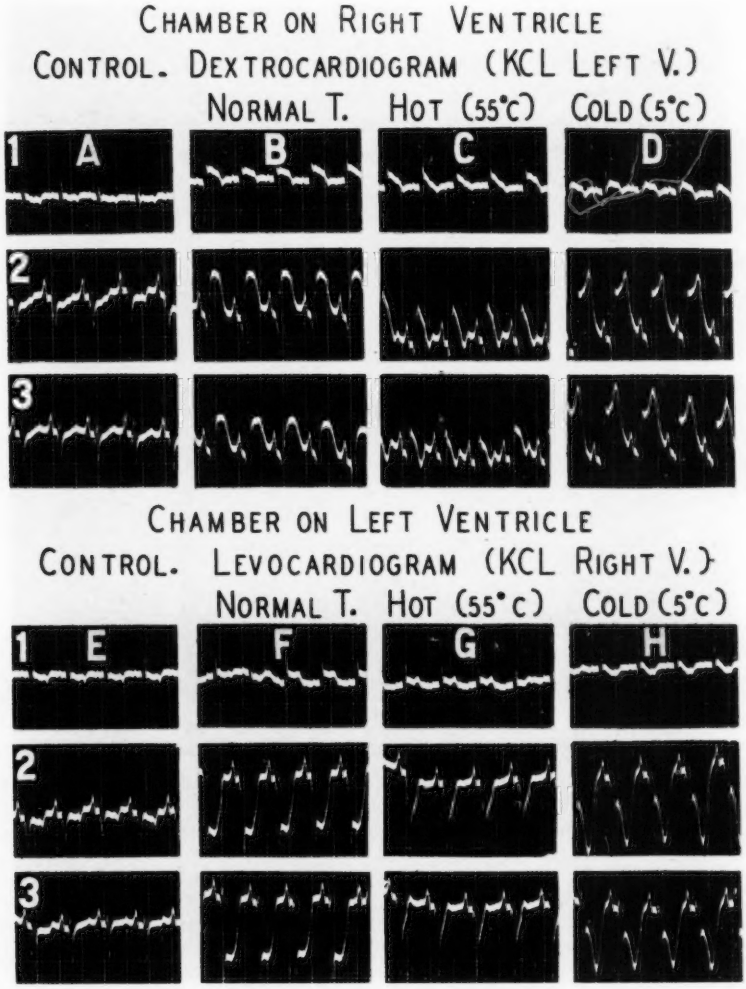

Fig. 4. Same experiment as figure 2. A. Control from three leads after all operative procedures, with chamber in place on the right ventricle at normal temperature. B. KCl pledgets were then placed over much of the left ventricle, giving a normal dextrocardiogram. In C the right ventricle was heated while the pledgets remained in place on the left ventricle. Records show shortening of the complex and disappearance of the plateau. In D the right ventricle was cooled. The dextrocardiogram was lengthened, and the plateau exaggerated. In E, F, G and H, the same series of experiments was carried out on opposite ventricles. The right ventricle was blocked with KCl to reveal the normal levocardiogram (F) which was affected by heat (G) and cold (H) exactly as was the dextrocardiogram.

Results. Results in all experiments were uniform. They were as follows:

a. Controls with the disk in place on each ventricle at body temperature, showed no appreciable changes in the electrocardiogram.

b. Cooling the surface of the left ventricle invariably produced a sharply inverted V-shaped T wave, with prolongation of the duration of Q-T

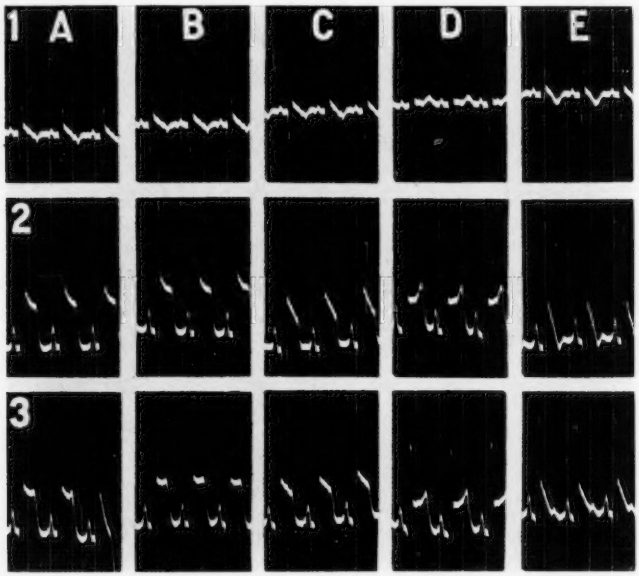

Fig. 5. June 8, 1940; 18.5 kgm. male dog. A. Dextrocardiogram produced by blocking left ventricles with KCl. The dextrocardiogram is best shown in leads 2 and 3. B. The region of the heart covered by the pledget was warmed by circulating water at 55°C through a chamber placed over the KCl pledget, which was of very thin filter paper. C. The region treated by KCl was cooled by circulating water at 5°C through the chamber. In neither B or C is there any alteration in the duration of the complex. In C there is a reduction in amplitude that is explained by the gradual decay of the potassium effect. In D and E the opposite ventricle (right) was cooled and heated respectively, producing the characteristic effect on the duration and contour of the complex. The same experiments carried out on the levocardiogram yielded identical results.

(fig. 2). Cooling the right ventricle produced an upright T wave which was also prolonged (fig. 2).

c. Heating the surface of the left ventricle produced an acutely upright T wave of normal duration, while heating the surface of the right ventricle produced a sharply inverted T wave of normal duration (fig. 2).

d. All areas of a given ventricle yielded qualitatively similar effects

when heated or cooled. Apical and basal areas acted similarly (fig. 3). This was confirmed further in two experiments where a smaller chamber was employed so that it was possible to investigate four separate areas on left ventricle, and three on the right ventricle. The only differences were in the magnitude of changes produced, and in the lead in which changes from the various areas were best recorded.

The nature of the dextro- and levocardiograms with heat and cold. To determine whether heating and cooling the surface of a single ventricle actually modifies the duration of the dextro- or levocardiogram as postulated, the separate ventricles were heated and cooled, while the opposite ventricle was blocked with KCl. Figure 4 summarizes the results, which were uniform in all experiments, and shows clearly that both the dextro- and levocardiograms were shortened materially by heat, and considerably lengthened by cold. In addition, changes in the contour of the complexes were produced.

As a control, the ventricle blocked by KCl was also heated and cooled, and this procedure did not alter the duration of the electrogram (fig. 5). These experiments confirm the hypothesis that the electrograms recorded after application of KCl to a single ventricle are the electrograms of the opposite untreated ventricle.

Discussion. The point of view which led to these experiments was first expressed by Burdon-Sanderson and Page, who showed clearly that heating the base of the tortoise heart caused an increased inversion of T. Since then several workers have employed the method, but continued the hypothesis that interference occurs between apex and base (Bayliss and Starling, 4; Mines, 5; Smith, 6). In 1909 Eppinger and Rotherberger froze the surfaces of the right and left ventricles with a spray of ethyl chloride, and noticed that in the first instance an upright T wave was produced, while in the second the T wave was inverted (7). In a previous paper from this laboratory it was shown that heat applied to the left ventricle at the apex produced an upright T wave, while cold caused an inversion (8). In the present experiments these observations are extended to both ventricles and an explanation of the T wave changes produced by heat and cold is given, based upon the influence of temperature upon the dextro- and levocardiograms.

It was predicted that since the T wave is produced by the interference of the terminal portions of the electrograms from right and left ventricles, modifications should occur in the T wave whenever the duration of these components is altered. In these experiments a single component was altered by applying heat and cold to the surface of a single ventricle. Heat actually shortened, and cold lengthened the dextro- or levocardiogram. When the dextrocardiogram was lengthened by cold, an upright prolonged T wave was obtained, and when the dextrocardiogram was

shortened by heat, a downward T wave was found, of normal duration. Lengthening the levocardiogram by cold produced a prolonged inverted T wave, and shortening the levocardiogram by heat gave rise to an upright T wave of normal duration. These changes were exactly as predicted theoretically.

These experiments afford additional proof that the interference which results in the normal electrocardiogram takes place between the two ventricles acting as units, and not between apex and base. In previous experiments (3) it was shown that application of small squares of filter paper soaked in M/5 KCl to various parts of the surface of a single ventricle always resulted in the displacement of the S-T segment in a single direction. In conformity with these results it is now seen that changing the temperature of various regions of the same ventricle always affected the direction of the T wave in the same manner.

It was clear from this study that the location on the heart of the area heated or cooled was an important determinant of the magnitude of the changes produced, and of the lead in which this change was best exhibited. This question will be reported in greater detail in another communication.

Further proof is here afforded that the electrograms we have recorded are in reality the electrograms of the ventricle not treated with potassium chloride, for heating and cooling the treated ventricle produced no changes in the recorded waves, while heating and cooling the opposite ventricle produced marked changes. These results can only mean that the electrocardiograms thus recorded are derived from the untreated ventricle.

These findings lead to the conclusion that alterations in the T wave result from variations in the duration of the electrograms of each ventricle. As seen in figure 4, variations in the contour of the dextro- and levocardiograms were also produced by changes in temperature. These must also be considered to have contributed to the final form of the T wave.

CONCLUSIONS

1. The T wave results from the interference of the terminal portions of the dextro- and levocardiograms.
2. Heat curtails the dextro- and levocardiogram.
3. Cold prolongs the dextro- or levocardiogram.
4. Prolongation of the dextrocardiogram or shortening of the levocardiogram causes an upright T wave.
5. Shortening of the dextrocardiogram or lengthening of the levocardiogram inverts the T wave.

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THE SKIN TEMPERATURE OF HYPERTENSIVE RABBITS AND THE PRESSOR EFFECTS OF HEATED KIDNEY EXTRACTS[1]

FREDERIC KAPP, C. K. FRIEDLAND AND E. M. LANDIS

From the Laboratory of Pharmacology and the Department of Medicine, University of Pennsylvania, Philadelphia, Pa.

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It has been shown previously in normal rabbits (1) that suitably heated kidney extracts elevate blood pressure conspicuously without diminishing blood flow to the ear, as indicated by the constancy of skin temperature during the entire pressor response. In sharp contrast, minute doses of epinephrine, tyramine, guanidine and pituitrin uniformly reduce peripheral blood flow whenever blood pressure is increased even slightly. Prinzmetal and Wilson (2) and Pickering (3) had found previously that in human hypertension peripheral blood flow in the extremities is approximately normal. Thus it appears that of the substances so far studied some component of kidney extract (presumably renin) is the only one which can imitate, though briefly, the circulatory changes characteristic of the hypertensive state in man. This interesting similarity made it seem desirable to extend these studies to rabbits made hypertensive by the Goldblatt method.

The experiments now described compare normal and hypertensive rabbits with reference to *a*, the initiation of peripheral vasodilatation by body warming; *b*, maximal skin temperature of the ear during complete vasodilatation as a rough measure of peripheral blood flow, and *c*, the effects of kidney extract with respect to the magnitude of the pressor response and the constancy of skin temperature during the period when blood pressure is elevated.

MATERIAL AND METHODS. All observations described here were carried out on male, white, New Zealand rabbits, weighing between 2.0 and 3.0 kilos, and fed on a standard adequate ration. To produce hypertension, small silver clips with a channel 0.4 to 0.6 mm. deep, were clamped about each renal artery through flank incisions. Before and after operation, blood pressures were measured in the central artery of the ear at weekly intervals by either or both of two methods; *a*, the recording oscil-

[1] The expenses of this study were defrayed in part by a grant from the Commonwealth Fund.

lometer previously described (1), or *b*, the simple capsule method developed by Grant and Rothschild (3). While blood pressure was being measured the vessels of the ear were kept widely dilated by raising the body temperature of the rabbit gradually to between 40.5 and 41.5°C. This was accomplished by wrapping an electric heating pad around the abdomen or, more usually, by placing the rabbit's body in a box with a double wall through which warm water (43–45°C.) was circulated. Rectal temperature and the skin temperature of the ear were both measured by the usual type of copper constantan thermal junction. The head and ears were exposed to room air at temperatures between 22° and 25°C.

Extracts of normal rabbit kidney tissue were prepared by suitable grinding, followed by heating to between 55 and 56°C. for twenty minutes, and subsequent filtration as described previously (1). These extracts were slowly injected intravenously in doses of 20 cc. at the rate of 1.6 cc. per minute for twelve minutes, through a minute T-cannula which had been stitched in a lateral ear vein at the beginning of the observation. It has been demonstrated (1) that with this technique salt solution or blood plasma could be injected into unanesthetized animals without disturbing skin temperature or blood pressure appreciably.

OBSERVATIONS. A. *Blood pressure levels in control and hypertensive animals.* Systolic blood pressures of normal animals observed as controls in these experiments generally ranged from 65 to 95 mm. of Hg; but in isolated animals readings as high as 103 mm. Hg were observed occasionally. After silver clips were applied to both renal arteries, approximately 60 per cent of the animals developed a sustained hypertension with systolic blood pressures between 110 and 176 mm. Hg; these will be referred to as "hypertensive animals." The silver clips used in these experiments were not adjustable so that if the clips, as originally applied, failed to produce hypertension the animals were necessarily discarded.

B. *Peripheral vasodilatation.* The vessels in the ears of normal and hypertensive rabbits responded similarly to changes in body temperature. When body temperature was between 37 and 39°C., the vessels of the ear were ordinarily constricted and the temperature of the skin at the tip of the ear approached that of the surrounding air (fig. 1). As body temperature was slowly raised the central artery of the ear dilated at first partially and spasmodically, but dilatation became complete when rectal temperature reached 40.5°C. or more. In a few minutes skin temperature reached a maximum and constant level which was lower than rectal temperature by between 1.5 and 4°C. This relationship held as long as the body temperature was kept above normal.

The larger section of figure 1 shows rectal and ear temperatures observed in a normal rabbit while body temperature was being slowly raised. The same figure could illustrate equally well the response of a hypertensive

rabbit to similar body warming. The inset in figure 1 shows ear temperature charted against rectal temperature. The solid line shows the average curve for ten normal rabbits; the dotted line shows the average curve for six hypertensive rabbits under similar conditions. At rectal temperatures

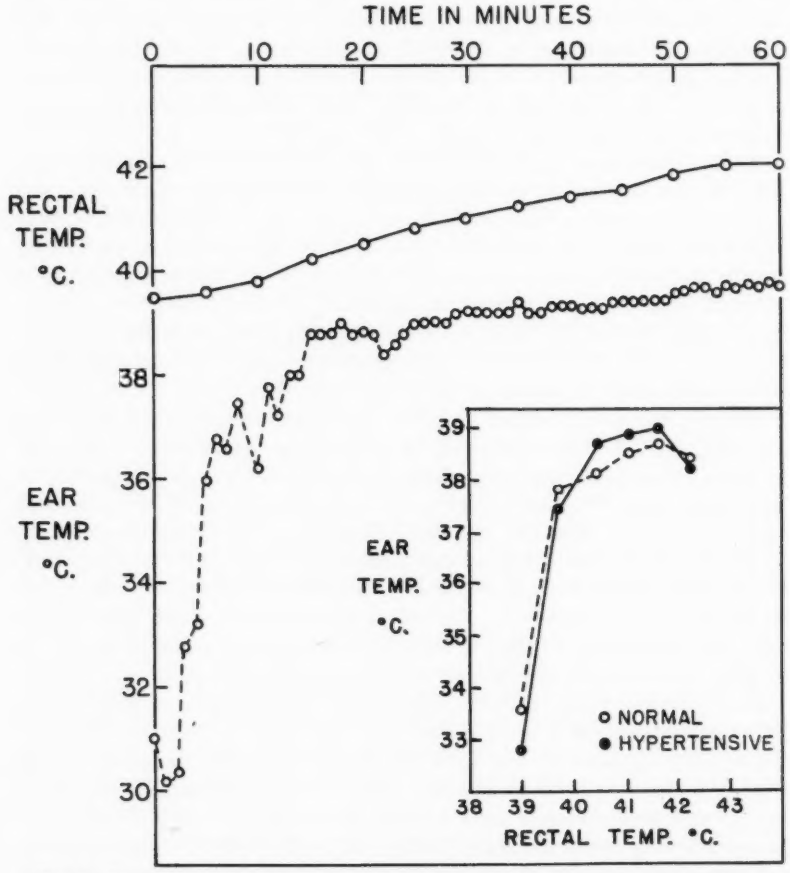

Fig. 1. Showing relationship of skin temperature of ear to rectal temperature in normal and hypertensive rabbits during body warming.

of 39.5° to 42°C. ear temperatures rose to similar maximum levels in the two groups, indicating that rabbits with experimental hypertension react normally to rising body temperature and that release of vasoconstrictor tone leads to a similar increase in peripheral blood flow. In this respect

a comparison of normal and hypertensive rabbits agrees with similar comparisons of peripheral blood flow in normal and hypertensive human subjects (2, 3).

The two curves are not identical, however, in that at rectal temperatures in the vicinity of 39°C. the average ear temperatures for the controls are slightly higher than those for the hypertensive animals. This is due to the fact that two of the hypertensive rabbits occasionally showed slightly delayed peripheral vasodilatation. The vessels did not relax until rectal temperature was between 40.3 and 40.7 instead of the more usual range between 39.5 and 40.3°C. Once dilatation began it rapidly became complete and the difference is so slight that it does not seem significant. Ill rabbits with normal blood pressure frequently show similar deviation from the normal reaction. In addition, it is possible that peripheral blood flow may actually be slightly greater than normal in the hypertensive animals during maximal vasodilatation. When rectal temperature was raised to between 40 and 41.5°C. the skin temperatures of the hypertensive rabbits were slightly higher than those of the normal rabbits under similar conditions. Thus, while it is safe to conclude that experimental hypertension in rabbits does not diminish blood flow to the ear, the method used does not exclude a slight increase in peripheral blood flow.

C. *The effects of kidney extract on blood pressure, skin temperatures and peripheral blood flow of hypertensive rabbits.* It has been shown (1) that the injection of a 10 per cent heated kidney extract into normal rabbits elevates systolic blood pressure by 40 to 45 mm. Hg without modifying skin temperature. Figure 2 illustrates a similar experiment using a hypertensive rabbit with a resting systolic blood pressure of 130 mm. Hg as the result of prior clamping of both renal arteries. The injection of 20 cc. of 10 per cent rabbit kidney extract into this animal elevated the blood pressure by over 50 mm. Hg, i.e., from 130 to 180 mm. Hg, also without modifying skin temperature. This result, typical of many additional observations, indicates that the pressor principle of kidney extract acts additively with the humoral mechanism of renal ischemia to elevate blood pressure still further. Both the persistent hypertension of renal ischemia and the added temporary elevation of blood pressure produced by kidney extract do not decrease peripheral blood flow measurably.

D. *Sensitivity of normal and hypertensive rabbits to kidney extracts of varying concentration.* It has been suggested frequently (5–7) that normal kidney tissue reduces the effect exerted by a given extract on the blood pressure of the recipient animal and, for this reason, nephrectomized animals have been recommended for assay purposes (5). In order to determine whether renal ischemia modified reactivity in rabbits, dilute and concentrated kidney extracts were injected into a series of normal and

hypertensive animals without anesthesia. Table 1 summarizes the results of this series of experiments.

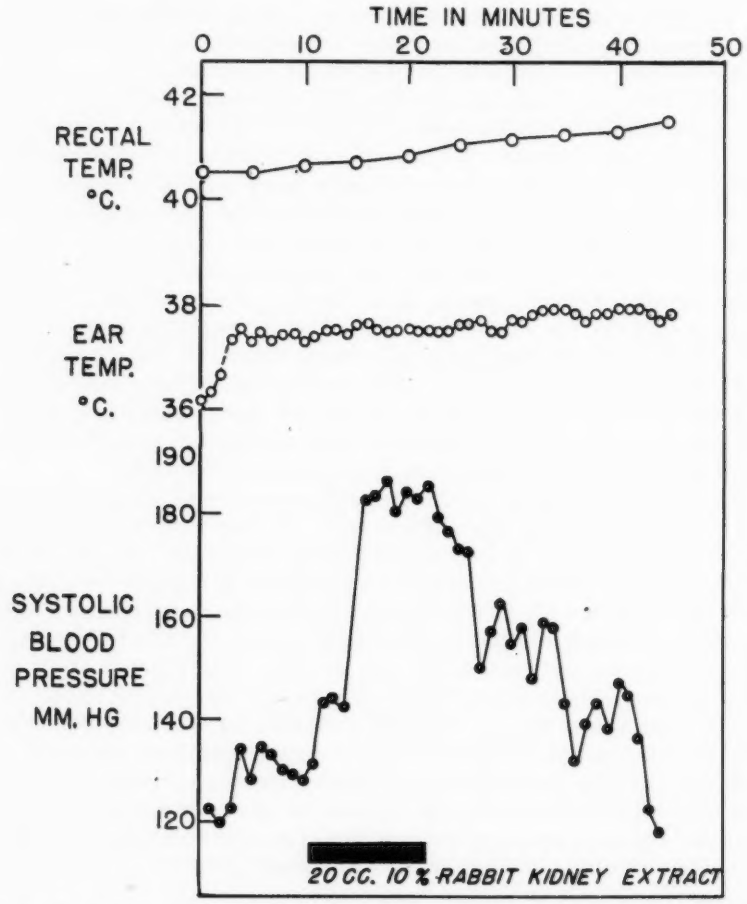

Fig. 2. Showing the effect of heated extract of normal kidney tissue of rabbit on systolic blood pressure and skin temperature during complete vasodilatation.

In neither group did blood pressure change significantly during the injection of 0.9 per cent NaCl solution and of 0.1 per cent heated rabbit kidney extract in the usual dose of 20 cc. injected at the rate of 1.6 cc. per minute over twelve minutes. Similar doses of 3.3 per cent rabbit

kidney extract elevated the blood pressure of the hypertensive animals more than that of normal animals, but when 10 per cent extracts were used the results became irregular, particularly in those animals with very high initial pressures.

TABLE 1

Showing changes in systolic blood pressure during injection of heated kidney extracts into normal and hypertensive rabbits

NORMAL ANIMALS			INJECTION: 20 CC. IN 12 MIN.	HYPERTENSIVE ANIMALS		
Blood pressure mm. Hg				Blood pressure mm. Hg		
Before injection	Maximum during injection	Difference		Before injection	Maximum during injection	Difference
78	78	0	NaCl 0.9%	134	132	−2
80	75	−5		110	110	0
90	89	−1		117	125	+8
75	78	+3		104	97	−7
70	70	0	Rabbit kidney	130	130	0
87	90	+3	extract 0.1%	110	112	+2
78	80	+2		116	119	+3
				115	116	+1
81	95	+14	Rabbit kidney	105	135	+30
77	85	+8	extract 3.3%	116	140	+24
84	100	+16		130	150	+20
83	138	+45	Rabbit kidney	127	188	+61
88	138	+50	extract 10.0%	117	149	+32
74	119	+45		106	150	+44
70	102	+32		128	184	+56
				176	188	+12

DISCUSSION. Substances which raise blood pressure may be classified into two groups according to their effect on peripheral blood flow in the unanesthetized rabbit. Nearly all of the common pressor substances resemble epinephrine in reducing cutaneous blood flow conspicuously when injected in the smallest doses which elevate systemic blood pressure measurably. So far only two pressor substances have been found which do not decrease cutaneous blood flow when raising systemic blood pressure. One of these, renin, is found in extracts of kidney tissue while the second, alpha-N-dimethyl-p-hydroxyphenylethylamine sulphate (paredrinol sulfate) is a synthetic sympathomimetic drug allied to ephedrine. The latter according to Stead and Kunkel (8) produces in man a type of "hypertension which has many features in common with clinical hypertension," one of these features being the sustained normal peripheral blood flow.

The present studies indicate that the hypertensive rabbit also maintains a normal peripheral blood flow despite conspicuous elevation of systemic blood pressure. This finding adds to evidence accumulating from other directions indicating that experimental hypertension produced in animals by renal ischemia truly simulates the human hypertensive state in which peripheral blood flow is also practically normal (2, 3). Moreover, heated kidney extract injected into animals already hypertensive, and more reactive than normal to renin, raised blood pressure to still higher levels without decreasing peripheral blood flow.

In hypertensive rabbits small doses of heated kidney extract elevate systolic blood pressure by slightly greater absolute amounts than is the case when similar doses are injected into normal animals. These results agree with those of Katz and Friedberg (6) for hypertensive dogs and of Williams, Wegria and Harrison (9) for hydronephrotic rats with hypertension. Concentrated extracts in three of five experiments on hypertensive rabbits raised systolic blood pressure to maxima of 188, 184 and 188 mm. Hg, whereas the absolute elevations of systolic blood pressure varied widely and were sometimes less than the elevations produced by the same extracts in normal animals. It is possible that the pressor effect of renal ischemia combined with that of the kidney extract approached the limit of the vascular system to respond and that this masked the greater sensitivity that could be demonstrated with more dilute extracts.

SUMMARY

The cutaneous vessels in the ears of hypertensive rabbits and normal rabbits responded similarly, both quantitatively and qualitatively, to body warming. The hypertensive state induced by renal ischemia was not associated with measurably diminished peripheral blood flow, indicating another resemblance between experimental hypertension in animals and the hypertensive state in man.

Heated kidney extracts, injected into hypertensive rabbits, raised blood pressure to extremely high levels likewise without diminishing skin temperature. Apparently the temporary pressor effect of kidney extract is added to the more permanent hypertension due to renal ischemia, without diminishing peripheral blood flow.

When the same kidney extracts were injected into normal and hypertensive rabbits, the rise in blood pressure was slightly greater in the hypertensive group, except that with large doses in markedly hypertensive animals there appeared to be a maximal value of systolic blood pressure which could not be exceeded. Under these circumstances the blood pressure of the hypertensive rabbits was increased by absolute amounts which were less than those observed with the same extract in normal rabbits.

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SOME AFFERENT DIENCEPHALIC PATHWAYS RELATED TO CORTICAL POTENTIALS IN THE CAT

E. W. DEMPSEY, R. S. MORISON AND B. R. MORISON

From the Departments of Physiology and Anatomy in the Harvard Medical School

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Electrical responses to afferent stimulation have recently been described in the cerebral cortex. These responses have been obtained after stimulation of the sciatic nerve (Derbyshire *et al.*, 1936; Forbes and Morison, 1939), the saphenous nerve (Heinbecker and Bartley, 1940) and tactile sensory organs (Marshall, Woolsey and Bard, 1937). At least three types of response may be distinguished on the basis of latency, threshold and localization in the cortex. In Marshall, Woolsey and Bard's observations on the monkey the latency was 8 to 10 msec., and the effect was sharply localized in the sensory cortex. Forbes and Morison (1939) obtained two cortical responses to stimulation of the cat's sciatic with single shocks. An initial "primary" response whose latency was 8 to 10 msec. was followed by a "secondary" response 30 to 80 msec. after the stimulus. The voltage of the primary response was greatest in the contralateral sensorimotor cortex, but the activity was not localized sharply under the conditions of their experiments. The secondary response was obtained equally well in all regions of the cortex, on both the contralateral and ipsilateral sides.

The effects described above are obtained best under such deep barbiturate anesthesia that only slight spontaneous cortical activity appears in the records. They may be recognized, however, when a moderate degree of spontaneous activity is present. Indeed, Heinbecker and Bartley (1940) described cortical responses whose latencies are similar after stimulation of the saphenous nerve in unanesthetized cats. In addition, they describe a third response of still longer latency (400 msec.) which occurs only after stimulation strong enough to activate the C fibers of the nerve.

The present investigation was undertaken to determine, if possible, the course and location of the afferent pathway or pathways to the cortex involved in the production of the primary and secondary responses. Data will be presented in the following sections which indicate that different pathways are involved for the two responses.

METHOD. Cats were anesthetized with a preliminary dose of nembutal (0.7 cc. per kgm.). Later, more nembutal was added intravenously until

good secondary responses were obtained. The cerebral cortices were exposed and one or both sciatic nerves were prepared for central stimulation. Wick electrodes, with an interpolar distance of 3 to 10 mm., were placed on various regions of the cortex. For more precise localization, bipolar silver wire electrodes with a separation distance of approximately 1 mm. were used in some cases.

The electrodes were connected with the input of 5 push-pull stages of condenser coupled amplification, and the responses were recorded by a Grass ink-writing electroencephalograph. In various experiments 1 to 5 independent channels of amplification and recording were used.

The sciatic nerves were stimulated by single condenser shocks, discharged through a thyratron tube which was controlled in turn by a manually operated key. The stimulus artifacts were regulated by an impedance balance consisting of a potentiometer shunted across the stimulus leads and connected to ground through the center tap.

Lesions, the exact nature of which will be described in the text, were produced by section, excision, or removal by means of a suction pipette of different parts of the brain. Responses were tested in all cases before and after the operation. Whenever any responses were abolished by the lesions, tests were made repeatedly at increasing intervals of time in order to determine whether or not the response was only temporarily depressed. In all, 50 experiments were performed.

At the end of each experiment the brain was removed and fixed in 10 per cent formalin. After sufficient hardening, gross sections were examined in order to determine the nature and location of the lesions. In certain experiments Nissl sections were prepared (fig. 3).

Results. In 21 experiments responses were tested in both cortices after stimulation of both sciatic nerves separately. "Primary" responses whose latencies were 8 to 10 msec. were routinely obtained in 9 of these experiments in which the recording electrodes were located on the leg area of the contralateral sensorimotor cortex (figs. 4 and 6). Small primary responses also were obtained on the ipsilateral cortex in some of these animals, while in others no recognizable ipsilateral "primary" could be distinguished.

In the remaining 12 experiments in which the sciatics were stimulated the recording electrodes were placed on other regions of the cortex. Under these conditions a small primary response was sometimes recorded, but it was never as large as those recorded from the sensorimotor leg area. For any electrode position, moreover, the primary response was larger on the contralateral than on the ipsilateral cortex. It appears, therefore, that the primary response is localized in the sensorimotor cortex, and that its magnitude is greater on the contralateral than on the ipsilateral side.

"Secondary" responses whose latencies were 30 to 80 msec. also were

recorded in all 21 of the experiments in which both sciatic nerves were stimulated. These responses occurred generally throughout both the contralateral and ipsilateral cortices. The latencies of the effects could vary, depending upon the region from which they were recorded. When simultaneous recordings were made from frontal, parietal and occipital areas, the latency in the occipital and parietal areas was equal to, or longer, but never shorter than that in the frontal cortex.

The stimulus threshold for both the primary and secondary responses was the same as that of A fibers in the sciatic, when tested oscillographically. Furthermore, both primary and secondary responses appeared simultaneously when thresholds were determined with 4 stimulating capacities ranging from 1.0 to 0.01 μ FD. Lastly, in an experiment in which electrodes were placed on an uncut sciatic nerve, the primary and secondary responses appeared with a stimulus intensity which was liminal for the motor nerve fibers. It appears, consequently, that the primary and secondary responses are produced by stimulation of nerve fibers of the same group.

The secondary response appeared simultaneously in both cortices after stimulation of either sciatic. There are, therefore, both crossed and uncrossed components in the afferent pathways. The following experiments were undertaken to delimit the levels of crossing.

Hemidecerebration at the intercollicular level was performed in 4 cats. After removal of 1 hemisphere, secondary responses were obtained from the remaining cortex, when either sciatic was stimulated (fig. 1). It may be inferred, therefore, that there is a crossed afferent component below the intercollicular level.

In 7 cats the brainstem was hemisected with a sharp spade at the intercollicular level. The hemispheres were not disturbed otherwise. Following the hemisection the primary response was abolished on the side of the lesion, while secondary responses were recorded from both cortices after stimulation of either sciatic in 4 animals. In the 3 remaining animals only one sciatic was stimulated. Secondary responses were recorded from both cortices in the 3 cases. These experiments indicate that a crossed afferent path is present above, as well as below, the intercollicular level.

The parietal and occipital cortex, diencephalon and midbrain were removed on one side in 6 cats. This preparation consisted essentially of hemidecerebration from the intercollicular level to the level of the optic chiasma but leaving intact the frontal cortex, the septal and preoptic areas and the anterior part of the corpus callosum. In all 6 experiments, secondary responses were recorded from both frontal cortices after stimulation of either sciatic, indicating that the crossed afferent component exists anterior to the level of the chiasm (fig. 2).

In 2 preparations of the type described above, the corpus callosum was

cut in addition to the cortical, diencephalic and mesencephalic lesion. The secondary response disappeared on the side of the defect, but was recorded on the opposite normal side in both cases. Conversely, in another preparation with the frontal cortex isolated but for its midline connections, the structures beneath the corpus callosum were divided in the midline by section with a sharp spade, care being taken to guide the spade along the inferior margin of the callosum. Following this procedure, secondary responses were recorded from both frontal cortices (fig. 2). Subsequent anatomical observation revealed that in this case the frontal cortex was indeed isolated except for its connections through the corpus callosum.

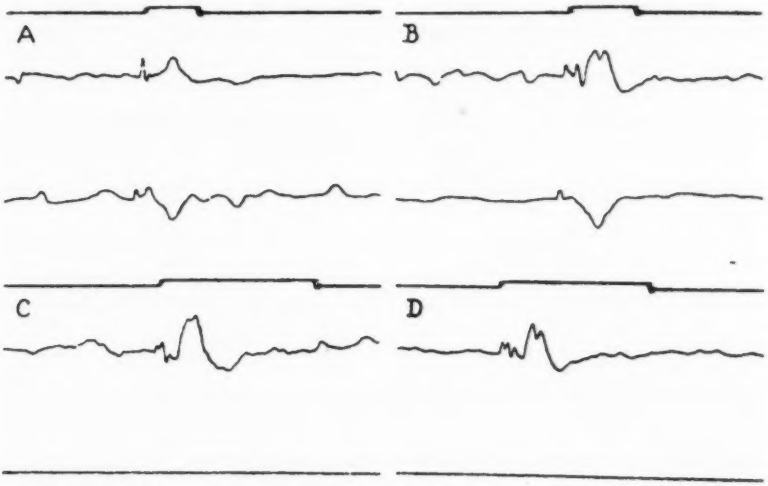

Fig. 1. Cortical responses to sciatic stimulation. Upper line, signal for stimulation; middle line, left sensorimotor cortex; lower line, right sensorimotor cortex. A and C, single shock stimulus to left sciatic; B and D, stimulus to right sciatic. Between B and C, the right hemisphere was removed from the intercollicular level forward. Paper speed—60 mm. per second.

In additional experiments on this point, the brainstem was hemisected at the intercollicular level and the corpus callosum was cut in 2 animals. Section of the callosum abolished the responses on the side of the hemisection, while secondary responses were recorded on the opposite, normal side. In another experiment the order in which the lesions were produced was reversed. The corpus callosum was first divided, and secondary responses were recorded in both cortices. Hemisection of the brainstem at the intercollicular level was then performed. After this lesion the responses were abolished on the side of the transection but were recorded on the opposite side.

Taken as a group, the experiments described in the preceding two paragraphs indicate that the upper crossed afferent path for the secondary response runs through the corpus callosum. Certain aspects of this pathway will be discussed in a separate communication. In order, however, to determine the course of the afferent path which is uncrossed above

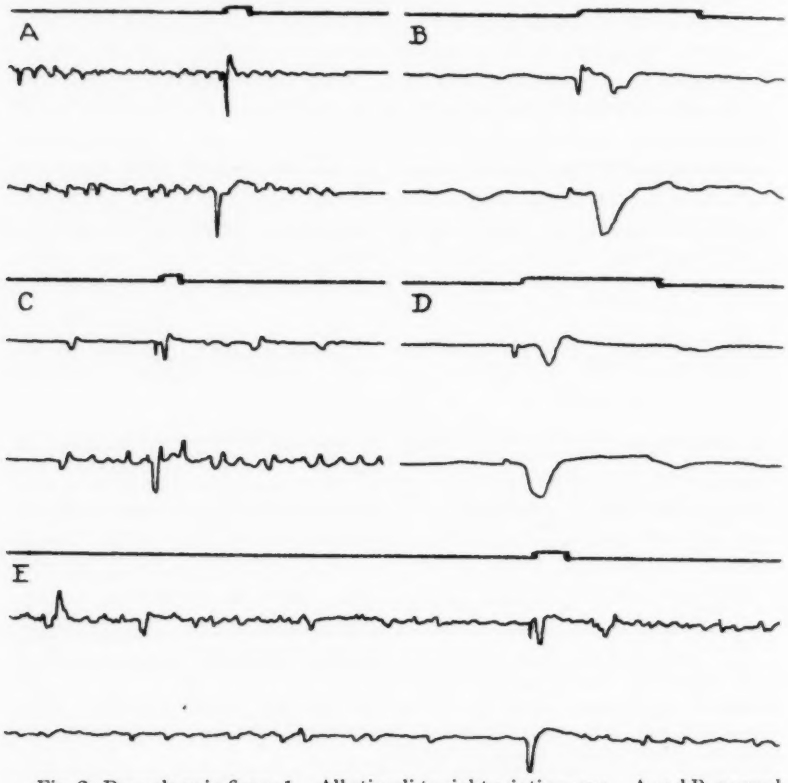

Fig. 2. Records as in figure 1. All stimuli to right sciatic nerve. A and B, normal brain. C and D, after left hemidecerebration, leaving intact the frontal cortex (see text). E, after midline sagittal section of all structures beneath the corpus callosum. Paper speeds, A, C and E, 15 mm. per sec. B and D, 60 mm. per sec.

the level of the colliculi, experiments were made in which unilateral defects were produced together with section of the corpus callosum, or in which bilaterally symmetrical lesions were placed.

In 5 cats the thalamus was removed or damaged extensively on one side (fig. 3). The operative procedure was used successfully to prepare chronic athalamic animals. A cut was made through the cortex into the ventricle,

2 to 5 mm. behind and parallel to the ansate sulcus. After identification of the lateral ventricle, the cortex overlying the thalamus was elevated and cut out. The cortical tissue which was removed consisted of those parts of the marginal gyrus, the gyrus fornicatus and the medial part of the suprasylvian gyrus which lie behind the anterior limit of the defect. The hippocampus and fornix, which were exposed by the cortical defect, were next removed, bringing into view the superior aspect of the thalamus.

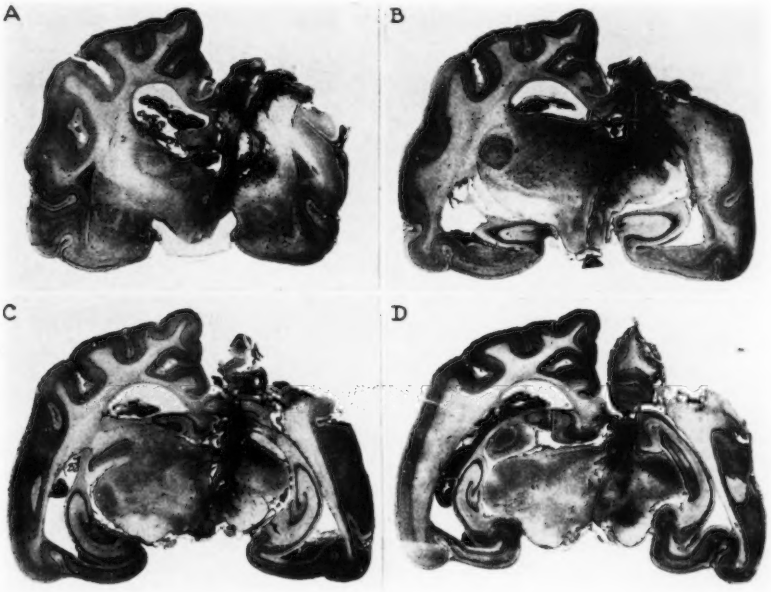

Fig. 3. Nissl sections through the level of the lesion in a cat from which the left thalamus had been removed three weeks previously. Cortical responses to sciatic stimulation in this animal are illustrated in figure 4. A, section through posterior border of optic chiasma and the anterior tubercle of the thalamus. B, section through the tuber cinereum and the anterior part of the lateral geniculate body. C, section through the mammillary bodies and posterior part of the lateral geniculate body. D, section through the red nucleus and the medial geniculate body.

Cuts were then made separating the massa intermedia in the midline and through the middle of the lateral geniculate body in the frontal plane. The body of the thalamus was removed by scooping or by suction. In all cases particular care was taken to remove the nucleus ventralis pars externa which is known (Ranson and Ingram, 1932) to receive fibers from the leg division of the medial lemniscus. Throughout the operative procedure close attention was given to hemostasis. Immediately after

the thalamic lesion the spontaneous electrical activity of the cortex was similar to that observed in chronic athalamic animals. Electrical stimulation of the motor cortex was followed by movements in most of the animals studied, indicating that the pyramidal system was still intact and functional.

Secondary cortical responses were recorded from both sides in these 5 unilaterally athalamic cats after stimulation of either sciatic (fig. 4, A

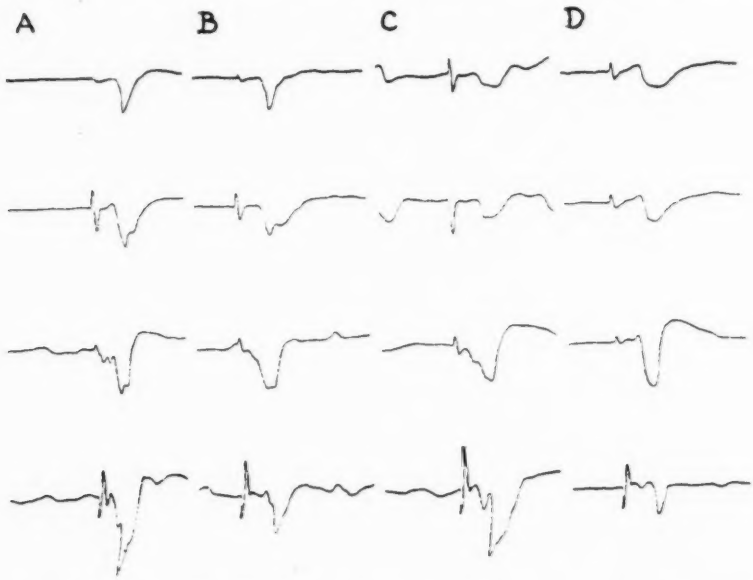

Fig. 4. Cortical responses after removal of thalamus. Records from above downward, left anterior sigmoid gyrus, left posterior sigmoid gyrus, right anterior sigmoid gyrus, right posterior sigmoid gyrus. Stimulations, A and C, left sciatic; B and D, right sciatic. The left thalamus had been removed three weeks previously (see fig. 3). Primary responses are seen in the right cortex, not in the left. Secondary responses are present in both cortices. Between B and C the corpus callosum was cut. Paper speed, 60 mm. per sec.

and B). In one of the experiments the recording electrodes were placed on the sensorimotor leg area in order to record both primary and secondary responses. After the operation, no primary could be detected in the record from the operated side.

The corpus callosum was then sectioned in these 5 animals. Bilateral secondary responses were recorded from 4 of the 5 (fig. 4, C and D). In the fifth, no response to sciatic stimulation could be detected on the operated side.

In 2 animals, after unilateral removal of the thalamus, the opposite thalamus was sucked out through the midline exposure. Secondary responses were recorded from both cortices in 1 of these animals, while in the other no cortical responses to stimulation were present. These experiments indicate that the uncrossed afferent pathway does not run through the thalamus, at least so far as the ventral, the medial or the anterior groups of nuclei are concerned.

Lesions also were made in the medial lemniscus at its entrance into the thalamus. The corpus callosum was sectioned and the lateral division of

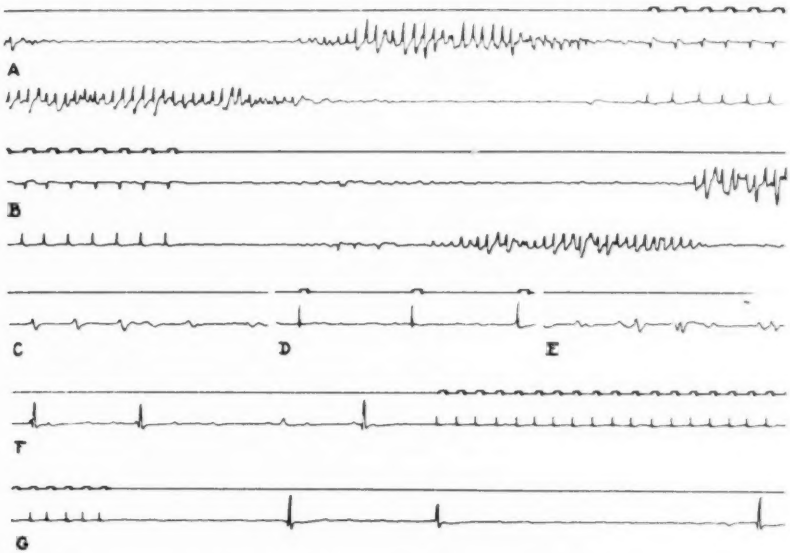

Fig. 5. Inhibition from sciatic stimulation. Records as in figure 1. Between A and B, 61 seconds of record removed. C, after bilateral cuts in the midbrain, sparing the central structures (see text). D and E, during and after sciatic stimulation. F, after application of strychnine to the cortical tissue. Between F and G 38 seconds of record removed. Paper speed, 7.5 mm. per sec.

the medial lemniscus cut on one side in 3 animals. Secondary responses were recorded from both cortices in 2 of these animals. In the third, no cortical response to sciatic stimulation was detected.

The lateral division of the medial lemniscus was sectioned bilaterally in 3 cats. Although no primary responses were seen in any of the 3 animals after the operation, the secondary effects were readily recorded in 2 cases. In the third, both primary and secondary responses were abolished after the section, but stimulation of either sciatic led to a suppression of the spontaneous and strychnine induced electrical activity of the cortex

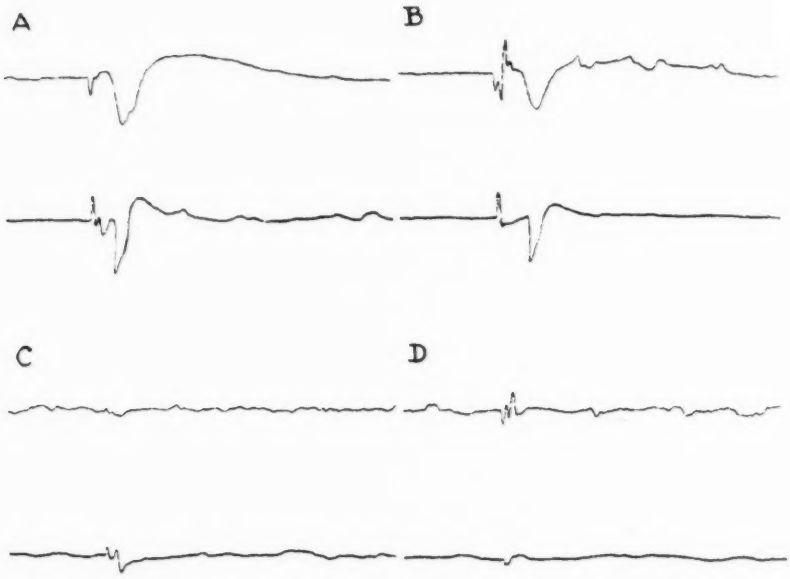

Fig. 6. Upper line, left posterior sigmoid gyrus; lower line, right posterior sigmoid gyrus. Stimulations, A and C, left sciatic; B and D, right sciatic. Both primary and secondary responses are seen. Between B and C, the lesion shown in figure 7 was placed. The primary response is still seen, but the secondary response has been abolished. Paper speed, 60 mm. per sec.

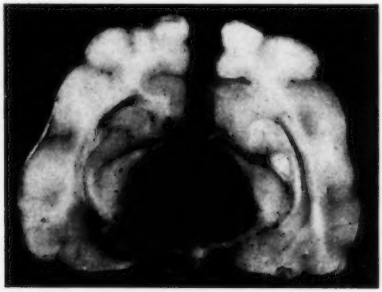

Fig. 7. Photograph of section through a midline lesion in the brain stem. Cortical responses to sciatic stimulation in this animal are illustrated in figure 6. The primary response was present and the secondary response was abolished after the lesion was placed.

(fig. 5). This inhibition was most marked when the sciatic was stimulated with a series of shocks at frequencies of 1 to 4 per second. Similar inhibition of spontaneous activity has been seen occasionally in normal animals after stimulation of the sciatic with slow frequencies (fig. 5).

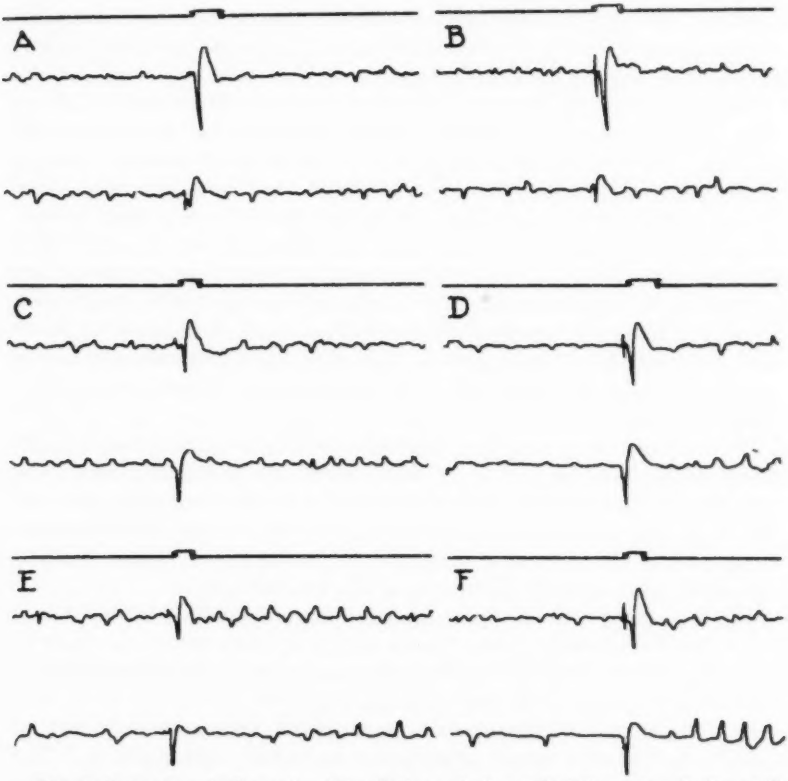

Fig. 8. Records as in figure 1. Stimulations, A, C and E, left sciatic; B, D and F, right sciatic. A and B, records from normal animal. C and D, after section of the cervical sympathetic nerves. E and F, after removal of the cerebellum. Paper speed, 15 mm. per sec.

The next type of experiment is the converse to that in which the medial lemniscus was sectioned bilaterally. In 4 cats a symmetrical cut was placed in the midline structures, beginning dorsally in the pretectal area and slanting slightly forward, to end ventrally at the level of the tuber cinereum. The lateral edges of the lesion involved the medial part of the

medial lemniscus but spared its lateral division which at this level has swung quite far outward as it enters the thalamus (fig. 7). In all 4 experiments good primary responses were present after the operation (fig. 6). In 3 of the 4, the secondary response was abolished by the lesion while in the fourth, both primary and secondary responses were obtained. Examination of the brain of this animal showed that the cut had been made further forward than had been intended and that the lesion was not entirely symmetrical in the subthalamic region.

In view of the long latency of the secondary response it was considered that it might represent vascular changes mediated by the sympathetic system. Another possibility was that it could be due to the activation of cerebellocortical connections. The cerebellum is known to receive spinal afferents, and the delay could be accounted for in the cerebellar circuits. The two possibilities were tested in the following experiment. Both cervical sympathetic nerves were sectioned in one animal, and secondary responses were subsequently recorded in both cortices after stimulation of either sciatic. The cerebellum was then excised. Secondary responses were still recorded in both cortices (fig. 8). The appearance of the secondary response, therefore, does not require either sympathetic or cerebellar circuits.

Discussion. At least three types of electrical responses may be produced in the cerebral cortex by stimulation of the sciatic nerve. The primary response appears with a latency of 8 to 10 msec. under the conditions of our experiments, and is localized in the leg area of the sensorimotor cortex. It is greatest in magnitude in the cortex contralateral to stimulation, but may also be present in the ipsilateral side.

The secondary response appears with a latency of 30 to 80 msec. and is not localized in any cortical region. Its latency, however, seems to be greater in the occipital than in the frontal cortex. These results are in agreement with those of Forbes and Morison (1939).

In addition to the above responses, inhibition of spontaneous cortical activity occurs after sciatic stimulation in certain circumstances. The exact conditions which are necessary for demonstration of this effect are not known. When present, inhibition is most prominent with stimulation frequencies which are fairly slow (1 to 4 per sec.).

These 3 cortical responses are not only different in character, but are produced by different afferent mechanisms. The primary response is abolished by lesions in the thalamus or lateral division of the medial lemniscus, while the secondary response remains after these procedures (fig. 4). Conversely, the secondary response is abolished by midline and subthalamic lesions at the level of the posterior border of the thalamus, while after these defects the primary response usually persists (fig. 6).

Finally, in the single experiment in which the brainstem had been damaged laterally on both sides, leaving intact only the central structures, inhibition of spontaneous activity could be demonstrated, although both primary and secondary responses had been abolished (fig. 5). It appears, therefore, that the afferent fibers for these three responses are to be found each in a different region, and that the responses may be selectively abolished by properly placed lesions.

The afferent to the primary response runs through the lateral part of the medial lemniscus, and projects to the leg area of the sensory cortex through the lateral nuclei of the thalamus. This conclusion is borne out by experiments (Morison, Dempsey and Morison, 1941) in which stimulation of the ventrolateral thalamic nuclei and the thalamic radiations produced cortical responses similar in all respects to the primary response. The latency and localization of this response are entirely similar to the potentials reported by Marshall, Woolsey and Bard (1937) after tactile stimulation. Moreover, since these regions are known to subserve functions of touch and proprioception, it is reasonable to conclude that the primary response is associated with these functions.

The course of the afferent fibers to the secondary response is less clear. The fibers do not run through the lateral part of the medial lemniscus or through the ventrolateral nuclei of the thalamus, since ablation of these structures does not abolish the response. In the experiments in which the secondary responses were abolished after thalamic lesions, anatomical inspection of the brains showed that the defect had involved the subthalamus as well as the thalamus. It is possible, therefore, that the afferent fibers run forward through the subthalamus. Stimulation of the subthalamus (Morison, Dempsey and Morison, 1941) is followed by secondary responses in the cortex, a fact which fits with this hypothesis.

The localization of the afferent fibers which inhibit cortical activity is obscure. At present, it can only be said that inhibition has been demonstrated after symmetrical lateral cuts in the midbrain which abolish both primary and secondary responses. It would appear, therefore, that the afferent fibers must run forward through the midline structures of the mesencephalon.

It was suggested by Forbes and Morison (1939) that the primary response might represent the arrival of the sensory impulse at the cortical level. This primary response, it was postulated, might then be regarded as the stimulus which sets off the secondary response. The failure of the secondary response to follow rapidly repeated stimulation could be due then to a decline in magnitude of the primary to subthreshold value, or to inhibitory effects which also were set up by sciatic stimulation. The present experiments, in as much as they demonstrate that the primary

and secondary responses are independent and are set off by different afferent pathways, render untenable the hypothesis that the primary response is the trigger for the secondary response. The failure of the secondary response to follow rapidly repeated stimuli must be due, then, to a long recovery process or to co-existing inhibition. That sciatic stimulation may inhibit both spontaneous and induced cortical activity is demonstrated in figure 5.

Heinbecker and Bartley (1940) have described cortical potentials after sensory stimulation in unanesthetized cats. The time relations of these potentials, which are similar to those of the primary and secondary responses, are attributed to conduction to the cortex over fast and slow fibers respectively. The long latency of the secondary response in our experiments cannot be a result of slow afferent conduction, since both the primary and secondary responses have the same stimulation threshold, which (Cf. Gasser and Erlanger, 1937, p. 41) indicates activation of fibers whose conduction velocity is the same. Furthermore, experiments in which the brainstem was stimulated (Morison, Dempsey and Morison, 1941) have shown that the secondary response can be elicited with practically unchanged latency when the stimulating electrodes are in the subthalamic areas.

CONCLUSIONS

1. Stimulation of the sciatic nerve in cats deeply anesthetized with nembutal may produce three cortical effects. These are: a primary electrical response whose latency is 8 to 10 msec. and which is localized in the leg sensorimotor area; a secondary response whose latency is 30 to 80 msec. and which may be recorded from any cortical area; and finally, inhibition of spontaneous cortical activity.

2. The primary response is abolished by lesions which destroy the thalamus or the lateral division of the medial lemniscus (fig. 4), while the secondary response remains after these procedures. It is concluded, therefore, that the former response is associated with functions of touch or proprioception.

3. The afferent supply to the secondary response is both crossed and uncrossed below and above the level of the colliculi. The upper crossed component runs through the corpus callosum (fig. 2). The uncrossed upper component is abolished by lesions which destroy the subthalamus (fig. 6), but the response remains after destruction of the thalamus (fig. 4).

4. Inhibition of cortical activity may occur after bilaterally symmetrical lesions in the midbrain which abolish both primary and secondary responses (fig. 5). The afferent pathway for inhibition runs, therefore, through the midline structures in the midbrain.

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CORTICAL RESPONSES FROM ELECTRICAL STIMULATION OF THE BRAIN STEM

R. S. MORISON, E. W. DEMPSEY AND B. R. MORISON

From the Departments of Anatomy and Physiology in the Harvard Medical School

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Cortical action potentials are produced by sensory stimulation under relatively deep barbiturate anesthesia (Derbyshire, Rempel, Forbes and Lambert, 1936; Marshall, Woolsey and Bard, 1937; Forbes and Morison, 1939, cf. for references). The cortical responses may be classified on the basis of latency and localization in the cortex. Different types of cortical responses may be selectively abolished by discrete lesions in the brain stem and diencephalon, and it has been suggested, therefore, that different afferent pathways are involved in the production of the various types of response (Dempsey, Morison and Morison, 1941). The present paper deals with the further tracing of these paths with stimulating electrodes introduced into the brain substance.

MATERIAL AND METHODS. Cats, anesthetized with nembutal, were used in this study. The skull was opened, exposing both cortices, and one or both sciatic nerves were prepared for stimulation. A modified Horsley-Clarke stereotactic instrument was attached to the skull and used as a carrier for the stimulating electrodes. This instrument, designed by one of us (R. S. M.) in conjunction with Dr. D. McK. Rioch, consists of a light aluminum frame attached to the edges of the skull at three points through universal joints, and an electrode carrier which allows for measured movements in three rectangular planes fitted to the frame (fig. 1). Although the device cannot be satisfactorily substituted for the conventional Horsley-Clarke apparatus when precise orientation of an electrode through a small bone defect is desired, it possesses compensating advantages. Its small size permits easy access to the exposed brain for the purpose of carrying out extensive operative procedures or the placement of a large number of cortical recording electrodes. The light weight (42 grams) leaves the head free to move easily in any plane without inducing additional sensory stimulation, and the absence of ear plugs permits the instrument to be used in the study of responses involving the autonomic nerves (to the orbit and salivary glands) which run through the middle ear. The coördinate readings do not determine the position of the electrodes with the definitive accuracy of the conventional instrument, but

with a little experience, an application of correction factors for variation in skull shape enables a comparable precision in use.

Recording electrodes, consisting of cotton wicks soaked in Ringer's solution, were connected by Ringer-agar-silver chloride junctions to silver wires. These wires led, in turn, to 5 push-pull stages of condenser coupled amplification. Recording was accomplished by a Grass ink-writing electroencephalograph. Two to five separate channels of amplification were used in various experiments.

Stimuli were single condenser discharges led to the stimulating electrodes through a transformer. The condenser discharges were regulated

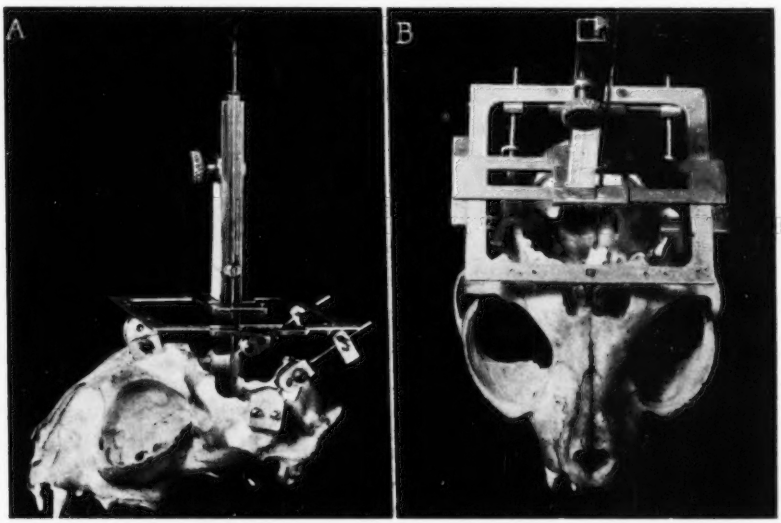

Fig. 1. Photographs of stereotactic instrument described in text, as applied to the skull of the cat.

by a thyratron tube which was controlled, in turn, by an external key. The stimulating electrodes consisted of 20-gauge steel tubing through which was placed an insulated copper wire. The steel tubing was covered, except at the tip, with an insulating layer of de Khotinsky cement. The center wire, also with bared tip, protruded about 1 mm. beyond the end of the tubing. Stimulation artifacts were controlled by a Wagner ground consisting of a potentiometer shunted across the stimulus and grounded at the center tap.

After preparation of the animal, more nembutal was injected intravenously until the base line of cortical activity was relatively stable and good secondary responses were obtained from sciatic stimulation (Forbes and

Morison, 1939). The stimulating electrodes were then introduced for systematic exploration of the brain stem and diencephalon. The explored region extended from the anterior commissure back through the level of the red nucleus. Because of the damage to the brain produced by the electrode tracks, all regions were not included in any single experiment, but overlapping regions were explored in different animals. In all, 1213 points were stimulated in the brains of 16 animals.

At the end of each experiment the brains were removed and fixed in formalin. For certain experiments Nissl sections were prepared for purposes of illustration. The shrinkage which occurs on embedding made it difficult to identify the points at which stimuli were applied. Consequently, in most of the experiments freehand sections of formalin-fixed material were made through the needle tracks, and the points were identified by gross measurements of the brains with reference to the coördinates of the stereotactic instrument.

Since the degree of localization provided by stimulation experiments is valid only in so far as it can be shown that the stimuli do not spread to regions remote from the electrodes, the following precautions against stimulus spread were taken. In the first experiments each point stimulated was explored routinely with stimulus intensities varying from zero to the maximum output of the thyratron stimulator. The threshold was determined for any response at any electrode position. The electrodes were then moved 1 mm. and the threshold for the same response again determined. In this way it was established that for each millimeter difference in electrode position there was a corresponding difference of approximately 20 points in the stimulus threshold. In all experiments the lowest threshold encountered was position 20 on the stimulus intensity dial.

In later experiments the procedure was modified as follows. The stimulus-intensity control was placed at position 30, corresponding to threshold plus 10 points. This position was chosen since it seemed likely that the stimulus spread would be confined to a sphere whose center was the electrode tip and whose radius was approximately one-half millimeter. Moving the electrodes downward millimeter by millimeter and stimulating at each point should therefore cover all regions in the electrode track and still permit localization to within 1 mm. In actual practice it appeared that this was accomplished. Responses obtained at one point usually were abolished by raising or lowering the electrodes 1 mm., and always were abolished when the electrodes were moved 2 mm. or more.

Results. Five types of cortical responses were produced regularly by stimulation in these experiments. These may be classified in the following manner. 1. A response whose latency is short (8 to 10 msec.) and which is localized more or less sharply in regions of the cortex on the side to which

stimuli were applied (fig. 2). 2. A fast response, similar to the one described above, which is succeeded by trains of spikes which are similar to the bursts of activity normally seen in the electroencephalogram of anesthetized animals (fig. 3). 3. A response of 30 to 80 msec. latency whose polarity is opposite to that of the prevailing spontaneous activity, and which may be recorded from all regions of both cortices (fig. 4, C).

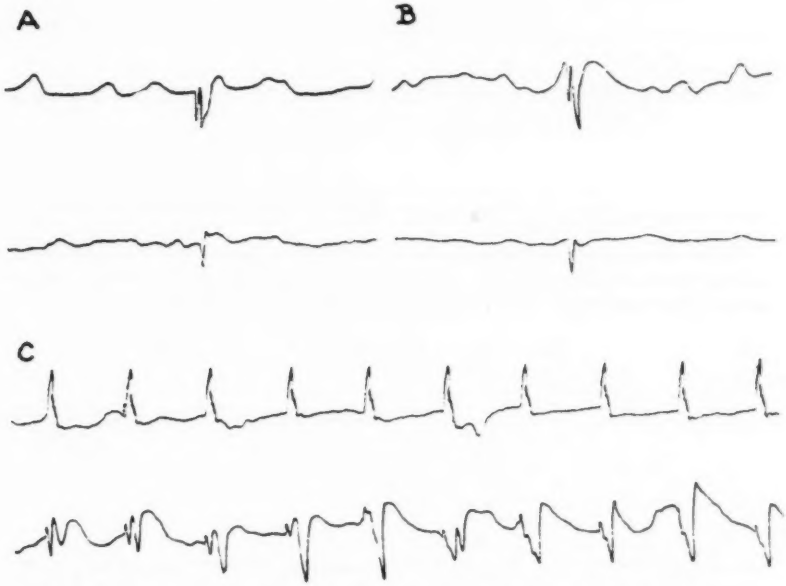

Fig. 2. Presence of short latency response ipsilateral to thalamic stimulation:

A. Electrograms of left (upper record) and right anterior sigmoid gyri. Shock artifact signals time of stimulation of left N. ventralis pars arcuata. The paper speed in this and succeeding records is 60 mm. per sec., unless otherwise noted.

B. Posterior sigmoid gyri of another preparation. Stimulation of left N. ventralis pars externa.

C. Recruitment of short latency response. Posterior sigmoid gyri of a third preparation. Stimulation of right N. ventralis pars externa at 5 per sec. Note: The upper tracing (left side) consists entirely of shock artifact.

This response is similar in all respects to the secondary discharge in the cortex after sciatic stimulation (Forbes and Morison, 1939; Dempsey, Morison and Morison, 1941). 4. A response which is similar in all respects to the secondary discharge, except that its latency is longer (100 to 250 msec., fig. 4, D). 5. Lastly, stimulation of certain areas in the brain leads to no visible action potentials, but rather causes the total or partial

suppression of the spontaneous activity which may be present at the time of stimulation (fig. 5).

A. *The short-latency, localized response.* The fast response (8 to 10 msec. latency) is encountered most frequently in the sensorimotor cortex and is seen only on the side to which stimuli are applied. It is produced by stimuli in the region of the lateral part of the medial lemniscus, in the ventral nuclei of the thalamus, and in the thalamic radiations (cf. figs. 2

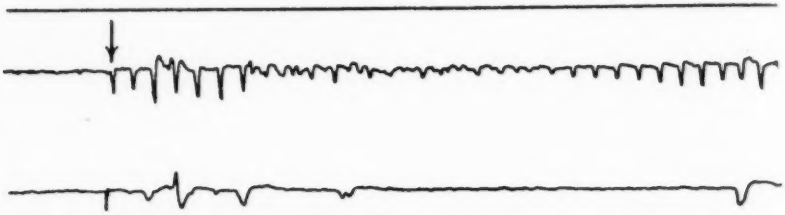

Fig. 3. Short latency response followed by repetitive bursts. Left (upper record) and right posterior sigmoid gyri. Arrow denotes single stimulus to anterior nuclear mass of thalamus (left). The activity on the right is spontaneous.

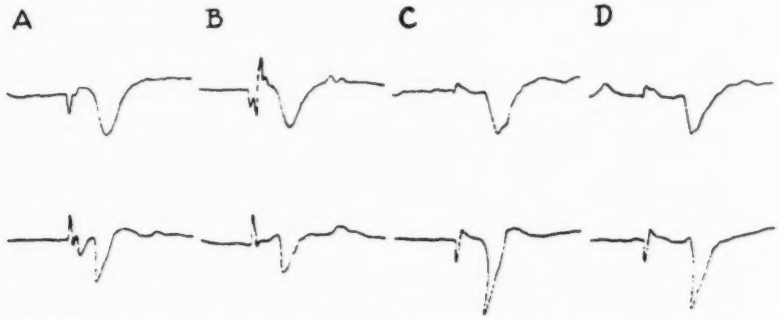

Fig. 4. Comparative latency of responses from different regions. Electrograms of left (upper record) and right posterior sigmoid gyri:

A. Left sciatic stimulated.

B. Right sciatic.

C. In region of Forel's field H, at level of mammillary bodies 5 mm. from midline. See figure 6 B.

D. Fornix (?) See text.

and 6). The localization of the response in the cortex is more or less sharp, depending upon the region stimulated. Stimulation of the substance of the thalamus tends to produce more discretely localized responses than does stimulation of the capsule or of the medial lemniscus. Repetition of the stimuli to form a series at a frequency of 1 to 4 per sec. occasionally led to an increase in the response suggesting a phenomenon akin to recruitment (fig. 2, C).

B. *The short-latency response followed by repetitive bursts of activity.* This response, like the preceding one, is most frequently seen in the sensorimotor cortex, and also is found only on the side of stimulation (fig. 3). It has been produced most frequently when the stimulating electrodes were placed in the anterior nucleus of the thalamus, the external medullary lamina, and the internal capsule.

The localization in the cortex and in the thalamus of the two types of response described above is admittedly incomplete at present. Indeed, the description given is undoubtedly an oversimplification, for the spike followed by bursts was on one occasion produced by stimulation of the ventral nucleus and stimulation of the anterior nucleus did not invariably

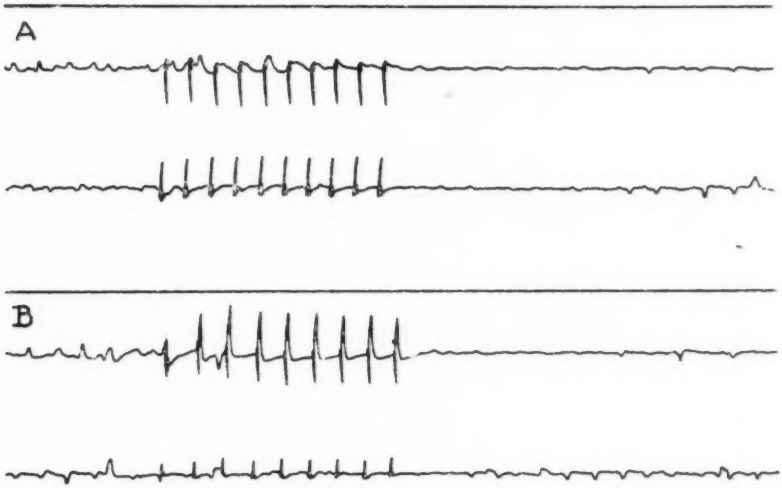

Fig. 5. Inhibition of spontaneous activity. Electrograms of left (upper record) and right posterior sigmoid gyri.

A. Stimuli to posterior part of head of left caudate.

B. Internal capsule slightly anterior to A.

produce the full response. The present results are included here because of their bearing upon the problem of the localization of the afferent pathways to the cortex which are involved in the production of primary and secondary responses after sciatic stimulation (Dempsey, Morison and Morison, 1941).

C. *The medium latency, generalized response.* Stimulation of the subthalamic regions is followed by a response in all parts of both cortices. The latency of this response varies from 30 to 80 msec. in different animals. In any animal the latency is identical or nearly identical with that of the secondary response to sciatic stimulation (fig. 4). The polarity, magnitude and duration of the response likewise are similar to these features of the

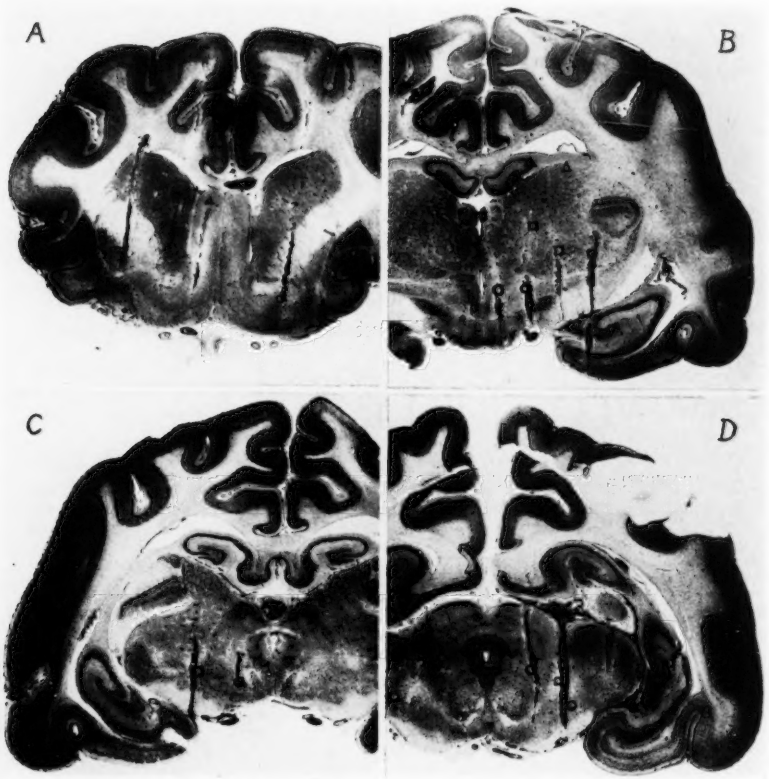

Fig. 6. Photomicrographs of Nissl sections of the cat's brain showing points from which responses similar to those illustrated in the accompanying figures were obtained. A, B, C from the same experiment: D, another preparation. Circles: Medium latency response (fig. 4 C). Triangles: Long latency response (fig. 4 D). Squares: Short latency response (fig. 2 A and B).

A. Section through the head of caudate, anterior part of anterior commissure and rostral pole of amygdala.

B. Section through habenular ganglia, and mammillary bodies.

C. Section through posterior commissure and habenulo-peduncular tract.

D. Section through large celled part of red nucleus. Stimulation of the points shown in this section frequently produced both short and medium latency responses, not illustrated but similar to 4 A and B.

secondary response. This generalized response has been obtained when the stimulating electrodes were placed in several points in the ventral part of the midbrain which contain bundles of fibers of the lemniscus system as they separate into medial and lateral components to enter

medial and lateral diencephalic areas (fig. 6, C and D). Slightly rostrally, responses were most consistently obtained in the region of the ventral part of the N. subparafascicularis, while at the level of the mammillary bodies active points were located in the region of the fields of H_1 and H_2 of Forel from 1 to 5 mm. from the midline, never more laterally and never so far dorsally as to include thalamic structures (fig. 6, B). Further rostrally, responses were less consistently obtained, but when they occurred the active areas were similarly located in the subthalamus.

The most rostral point which gave responses (4 out of 7 expts.) was situated at approximately the level of figure 6, A and was found to lie just ventral to the anterior part of the anterior commissure (5–7 mm. from the midline). Of the many nuclei and fiber tracts in this region, the medial part of the rostral pole of the amygdaloid complex was apparently most closely related to the stimulating electrodes.

D. *The long-latency, generalized response.* A response whose latency is 100 to 250 msec., and whose polarity and wave form are similar to those of the medium latency response, occurred after simulation of certain areas in the brain. This response is indistinguishable from the medium latency response and from the secondary response to sciatic stimulation, except for its latency (fig. 4, D). It has been obtained from the cortical grey and the associated white matter of the cingular gyrus, and also when the electrode tip, as judged by comparisons of the recording of the depth scale with the sectioned brain, was in the corpus callosum or the immediately subjoined fibers of the fornix system (fig. 6, A and B). Since this part of the brain is easily distorted by the pressure of the needle, and there is grave danger of spread of current in the ventricular fluid, it is difficult to be certain whether the fornix as well as the cortex was involved. Future stimulations under direct vision and with the ventricle emptied of fluid may help to elucidate this point.

E. *The inhibition of spontaneous activity.* In several experiments stimulation decreased the spontaneous activity of the cortex. This effect was produced best by a series of stimuli at a frequency of 1 to 4 per second, although it could be demonstrated occasionally after single shocks. Frequently there was an after-discharge of inhibition which outlasted the stimulation by several seconds (fig. 5). Inhibition was not tested routinely, since in the majority of the experiments only the responses to single shocks were observed. Nevertheless, inhibition has been seen when the stimulating electrodes were placed in the caudate nucleus (cf. Dusser de Barenne and McCulloch, 1938b, for the similar effect of strychninization of the same area), in the associated parts of the internal capsule, and even in the corona radiata above the corpus callosum. When the internal capsule was activated the inhibition was confined to the cortex on the side stimulated, while in the other experiments inhibition of activity in both cortices resulted.

Discussion. The experiments described in the preceding sections were undertaken because of their bearing upon the localization of the afferent pathways involved in the production of primary and secondary cortical discharges after sensory stimulation.

It has been shown elsewhere that these responses to sensory stimulation can be selectively abolished by properly placed lesions (Dempsey, Morison and Morison, 1941). The question arises, therefore, whether the results from stimulation of points in the brain can be homologized with the results obtained from stimulation of sensory nerves.

The primary response to sciatic stimulation has a short latency (8 to 10 msec.), is sharply localized in the leg sensorimotor area on the side opposite to the stimulus, and is abolished by lesions which involve the lateral part of the medial lemniscus or the ventrolateral nuclei of the thalamus (Dempsey, Morison and Morison, 1941). In the experiments presented here it is shown that stimulation of the ventrolateral thalamic nuclei, the lateral part of the medial lemniscus, or the thalamic radiations is followed by a fast response in the ipsilateral sensorimotor cortex. It appears, therefore, that the responses to contralateral sciatic stimulation and to ipsilateral thalamic stimulation may both be due to activation of the same cerebral pathway. It is hardly necessary to point out that this path coincides with the well-known course of somatic sensation as described by classical anatomy. Reference may also be made here to the discussion by Dempsey et al. (1941) of the similarity of this response to the "primary" response of Forbes and Morison and the cortical activation by touch stimulation of Marshall, Woolsey and Bard (1937).

It should be emphasized that in the present investigation no systematic study of the localization in the cortex of the representation of specific parts of the ventrolateral nuclear mass of the thalamus has been attempted. It seems probable, however, that the method employed will reveal discrete representation of face, leg, and arm areas in the thalamus of the cat as has been shown anatomically by a number of authors (cf. Walker, 1938) and physiologically (Dusser de Barenne and McCulloch, 1938a) for primates. The further possibility that the cortical representation of ventralis pars arcuata may differ from that of pars externa, as has been suggested by Walker for the homologous nuclei of primates, remains to be investigated.

There are also essential similarities between the secondary response to sciatic stimulation and the medium-latency generalized response. Both occur at the same latency and both have the same appearance with regard to polarity, voltage and duration. The secondary response is abolished by lesions which destroy the subthalamic regions, and the medium-latency response occurs after stimulation of these regions. It appears, therefore,

that here, too, we are dealing with activation of the same pathways after either sciatic or central stimulation.

Just what fibers form the pathway for the secondary response is difficult to determine. Presumably at midbrain and lower diencephalic levels they are represented by the medial divisions of the medial lemniscus which have a roughly similar course (cf. Rioch, 1931). Cajal (1911) indeed has followed collaterals into the zona incerta, a course exceedingly similar to that required by these experiments. Although good responses were obtained in the region of the thalamic fasciculus of Forel, it is unlikely that the dento-rubro-thalamic tract is involved since the presence of the dentate nuclei (Dempsey et al., 1941) is not essential for responses to sciatic stimulation, and stimulation of the terminal nucleus of the tract (ventralis pars arcuata (Ranson and Ingram, 1932)) does not produce the phenomenon. Indirect somatic afferent connections with the basal telencephalic grey situated in the region of our most rostral active points have been suggested by various authors but have no very well recognized status. For the present it may only be asserted that impulses traveling probably over the medial division of the medial lemniscus course through the dorsal and medial part of the subthalamus to the region of the anterior pole of the amygdala, and thence are widely distributed to the cortex (Morison, Dempsey and Morison, 1941). Judged from the very small difference in latency between responses elicited by stimulation anywhere along this path, conduction through the entire system from sciatic to basal telencephalon must be very rapid. This suggests that a minimum of internuncial neurons is involved.

The fast response without the medium-latency response occurs after stimulation of the ventrolateral nuclei of the thalamus (fig. 2), and the medium-latency effect is produced without the fast effect when stimuli are applied in the region of the fields of Forel (fig. 4) or rostral thereto. These two responses are therefore completely separable in stimulation experiments. Similarly, in lesion experiments selective abolition of the primary and secondary responses to sensory stimulation was produced (Dempsey, Morison and Morison, 1941). It appears abundantly clear, therefore, that the two components in the cortical response to sensory stimulation occur as a result of activation of two separate and distinct afferent pathways, at least beyond the level of the midbrain.

The long-latency generalized response appears to be identical with the medium-latency response in all respects except latency. Since the latency is longer than that observed after sensory stimulation, it is clear that the regions from which the slow response is elicited cannot represent parts of the afferent pathway from the sciatic. The similarities in the wave forms are so great, however, that they suggest that the same final response may

be produced by activation from different afferents. If this hypothesis be correct, the long latency should represent a slower conduction rate in its afferent pathway. It should be noted, however, that the thresholds for both long and medium latency responses are the same, and that these thresholds are also the same as those of mammalian A fibers.

SUMMARY

The brain stems of cats have been systematically explored with single stimuli applied through discrete electrodes, while recording cortical activity in 2 to 5 regions of the cerebral cortex. Five types of cortical response have been observed. These responses are as follows:

1. A response, which has a latency of 8 to 10 msec. and which is localized in the cortex of the side ipsilateral to stimulation, is produced by stimulation of the medial lemniscus, the ventrolateral nuclei of the thalamus, the thalamic radiations and the internal capsule (figs. 2 and 6). Reasons are given which indicate the identity of this response with the primary response to sensory stimulation (p. 740).

2. A fast response, which resembles that described above but which is followed by bursts of spikes similar to the spontaneous bursts of activity seen in the electrocorticogram from anesthetized animals, is produced by stimulation of the anterior nucleus of the thalamus, the thalamic radiations and the internal capsule (fig. 3).

3. A medium-latency (30 to 80 msec.) generalized response is produced in all regions of both cortices by stimulation in the region of the rostral pole of the amygdaloid complex, the dorsal part of the subthalamus, and the region of the nucleus subparafascicularis (figs. 4 and 6). This response is identical in wave form and nearly so in latency to the secondary response produced by sensory stimulation (p. 740).

4. A long-latency (100 to 250 msec.) generalized response which, except for latency, is identical with the medium-latency response, is produced by stimulation of the fornix, the corpus callosum, the radiations to the cingular and the suprasylvian gyri (figs. 4 C and 6). The possibility is discussed that this response represents activation of a slower afferent path to the response described in 3 above (p. 741).

5. Inhibition of spontaneous cortical activity may be produced by stimulation of the caudate nucleus, the internal capsule and the cortical radiations (fig. 5).

The bearing of these results on the localization of afferent pathways to the cortex from sensory stimulation is discussed (p. 740).

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ON THE PROPAGATION OF CERTAIN CORTICAL POTENTIALS

R. S. MORISON, E. W. DEMPSEY AND B. R. MORISON

From the Departments of Anatomy and Physiology, Harvard Medical School

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Previous studies (cf. Dempsey, Morison and Morison, 1941) have shown that sensory stimulation gives rise, under certain conditions, to a widespread cortical discharge with a latency of from 30 to 80 msec. The presence of the thalamic nuclei is not necessary for the response, but subthalamic and apparently basal telencephalic mechanisms participate in its production. Further interest attaches to the phenomenon since its general form and distribution are entirely similar to those of the most prominent spontaneous cortical waves which occur either under deep barbiturate anesthesia or in animals in which the corticothalamic circuits responsible for the more usual cortical rhythms (Dusser de Barenne and McCulloch, 1938) have been interrupted. Forbes and Morison (1939) have shown that the response is no longer obtainable from the remaining parietal and occipital cortices after removal of all the tissue anterior to the level of the junction of the anterior and middle thirds of the lateral gyrus. They left unanswered, however, the question whether or not the frontal cortical substance itself is essential for the firing of the remaining cortex. As they recognized, their lesion might just as well have interrupted direct paths from basal regions which normally distribute the activity more or less simultaneously to all parts of the cortex. The principal question considered in this paper therefore is: does the widespread nature of this response reflect the activity of some definite cortical "trigger zone" spreading through short neuron chains of association to other cortical areas, or does it depend upon more or less direct paths from subcortical areas to all parts of the cortex irrespective of their connections with one another?

The problem may be conveniently separated into two divisions, one dealing with the spread of activity from one hemisphere to the other (the crossed response) and a second concerned with the transmission within one hemisphere.

METHODS. The methods and apparatus used were similar to those described in accompanying papers (Dempsey et al., 1941; and Morison et al., 1941) and need not be discussed here.

RESULTS. A. *Spread of activity from one hemisphere to the other, "the*

crossed response." In a previous paper (Dempsey, Morison and Morison, 1941), it was demonstrated that after hemisection or removal of various parts of the midbrain and diencephalon, sciatic stimulation still elicited a secondary discharge from both cortices. The activity in the cortex ipsilateral to the lesion, designated as the crossed response, was found to depend upon the integrity of the corpus callosum (loc. cit., p. 721).

Since the chief function of the latter structure is ordinarily believed to be the association of homologous points in the two cortices (cf. the trans-

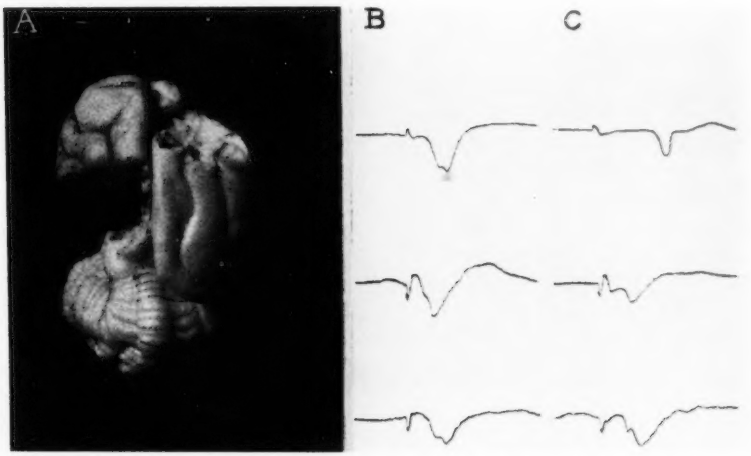

Fig. 1. A. Photograph of cat's brain after removal of cortical and basal areas on left, sparing the frontal cortex, and of the right anterior pole, sparing the fibers of the corpus callosum. Electrograms: records from above downwards: left anterior sigmoid gyrus; right anterior sigmoid gyrus; right posterior ectosylvian gyrus. Paper speed in this and succeeding records, 60 mm. per sec., unless otherwise indicated. B. After sciatic stimulation (at artifact), after production of the lesion on the left. C. Same, after removal of the right frontal pole. In this record the right posterior sigmoid gyrus electrodes were replaced on the edge of the lesion.

mission of strychnine spikes) (Gozzano, 1936) and of electrically induced activity (Curtis and Bard, 1940), the following experiment was done.

In two preparations in which all cortical and basal structures between the anterior margin of the thalamus and the colliculi had been removed on the left side, the right cortex anterior to this level was removed by a sloping cut sparing the anterior third of the callosum. Responses to sciatic stimulation were still present in all parts of the remaining cortex even though the homologous opposite portions were absent (fig. 1). Further evidence that the callosal fibers responsible for the crossed response form a rather definite bundle different from those connecting symmetrical points was

derived from the following type of experiment. The brain stem was hemisected at the intercollicular level and the ipsilateral cortex explored for responses after section of various parts of the callosum. Responses were found to be present over the entire cortex with as much as the posterior two-thirds of the callosum divided. Conversely, in other experiments, section of merely the anterior third wholly abolished the crossed response.

B. *Spread of activity within a single hemisphere.* The observation by Forbes and Morison (1939) that removal of all tissue frontal to a perpendicular plane at the posterior border of the anterior third of the lateral

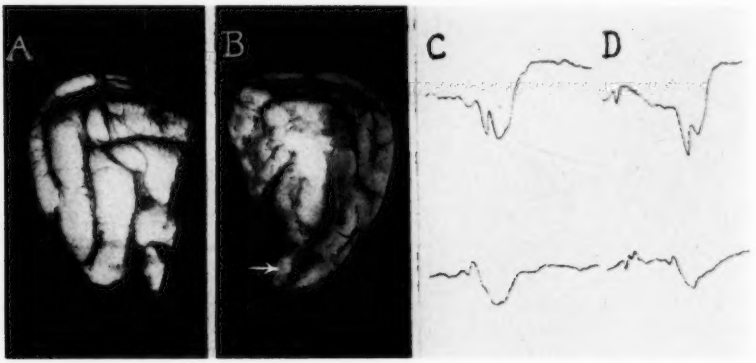

Fig. 2. Photographs of right cortical remnant deprived of all its connections with the rest of the brain except for the small bit of white matter, indicated by arrow, at the tip of the temporal horn of the ventricle. A. Lateral view. B. Medial view. C. Electrogram: upper line, left posterior ectosylvian gyrus; lower line, right conjugate cortex. The spontaneous activity from the cortex represented in 2 A and B is synchronous with that in the opposite normal cortex. D. Electrogram: secondary responses to sciatic stimulation in the normal left cortex (upper line) and right occipital cortex shown in A and B (lower line) are shown.

gyrus abolished the response in the remaining cortex was confirmed. Similarly, in preparations with midbrain hemisections, the preceding operation, though performed only on the contralateral side, abolished the response in all the remaining cortex. It is important to point out, however, that removal of similar amounts of neocortex without damage to basal structures did not affect the response in any remaining cortex (cf. fig. 1). In fact, the response was still present over the entire cortical area illustrated in figure 2 after severance of all its connections with the rest of the brain save for a small group of fibers in the region of the temporal horn of the ventricle (fig. 2, B). These experiments constitute strong evidence that the response in the occipital and parietal cortex does not depend upon the presence of the more frontally situated cortical areas

and is therefore not cortico-cortical. On the other hand, a relatively localized stab wound in the region spared by the preceding type of operation abolished the response over a wide area of the homolateral occipital cortex (figs. 3, A and C; 4, A and B). It might be objected that the disappearance of the response in the latter type of experiment was due to interference with the blood supply or to other nonspecific factors. Such an interpretation is inadmissible. Great care was taken to avoid severing major arteries when making the operation, and the cortical vessels remained well filled after the procedure which was indeed much less extreme than many other operations without effect on the responses. Furthermore, the application of strychnine to a small part of either cortex resulted in typical strychnine "spikes" which were transmitted to the opposite conjugate part of the cortex (fig. 3, D and E). Not only did this establish the fact that the operated cortex was not seriously injured, but it emphasized that the secondary discharge to sciatic stimulation involves cortical elements which are different from those involved in the strychnine spikes, since only the latter were capable of setting up a contralateral response in these conditions.

The discovery that the impulses responsible for the secondary discharge travel up from the brain stem and spread over apparently direct paths to the cortex from an area in the region of the temporal horn of the ventricle raised a further question concerning the crossed response (see section A). Do the fibers which carry the response across the callosum run directly to the various cortical areas, or do they first run down to the opposite temporal horn and make connection with the distributing system which arises there? The following experiment was therefore undertaken. The mid-brain was hemisected as in the previous experiment (p. 746) and a stab wound made on the *same* side far lateral and deep in the temporal lobe similar to that made contralaterally in other preparations. This lesion prevented the response of a corresponding part of the ipsilateral occipital cortex. Another lesion anteriorly placed but still far lateral in the cortex abolished the response in the frontal cortex. Subsequent unilateral application of strychnine to small cortical areas demonstrated the intactness of the known callosal cortical association fibers (fig. 3, E). The conclusion seems unescapable that the crossed response does not reach the opposite cortex directly through cortico-cortical association bundles, but first makes connection with structures deep in the temporal lobe and is thence distributed to the cortex of that side.

C. *Effects of the foregoing lesions on a component of the spontaneous cortical activity.* It has previously been pointed out (Forbes and Morison, 1939) that under deep nembutal anesthesia the spontaneous cortical activity may be almost completely reduced to a series of widely separated waves each of which bears a striking resemblance to the secondary dis-

charge elicited by sciatic stimulation. The simultaneous recording of various widely separated cortical areas in the present study showed that this activity occurred at nearly the same time throughout the cortex as long as the conducting systems outlined in sections A and B of this paper remained intact. Slight differences in the time of occurrence of the ac-

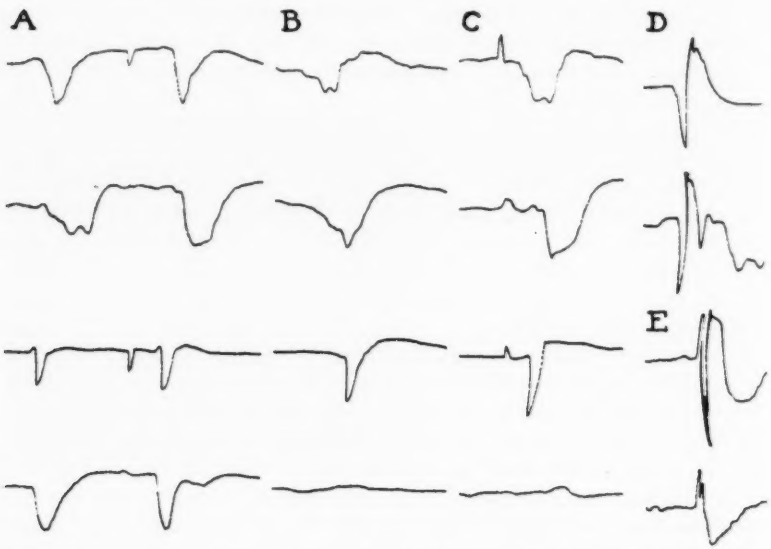

Fig. 3. Dependence of certain types of activity upon the integrity of fiber system arising near temporal horn of ventricle. See text. Electrograms from above downwards: left anterior sigmoid gyrus; left posterior ectosylvian gyrus; right anterior sigmoid gyrus; and right posterior ectosylvian gyrus. A. Spontaneous activity and secondary response to sciatic stimulation. B. Spontaneous activity after the lesion illustrated in figure 4 had been produced. C. Secondary response to sciatic stimulation after the lesion shown in figure 4. D. Strychnine "spike" induced by application of strychnine on the left posterior ectosylvian gyrus (lower record) and transmitted to the normal opposite conjugate cortex (upper record). E. Another preparation. Strychnine "spike" transmitted from right posterior ectosylvian gyrus (upper record) to opposite conjugate cortex after complete abolition of secondary responses and spontaneous activity by a lesion in the region of the right temporal horn of the ventricle.

tivity in various parts of the cortex of one hemisphere and somewhat longer intervals between its appearance on the two sides (either side might lead) are apparently explicable as the time necessary for conduction, but within these limits the activity may be referred to as synchronous throughout the cortex. Especially noteworthy is the fact that this type of activity

was particularly striking in preparations in which extensive damage had been done to the thalamus either acutely or some months previously (fig. 5, A).

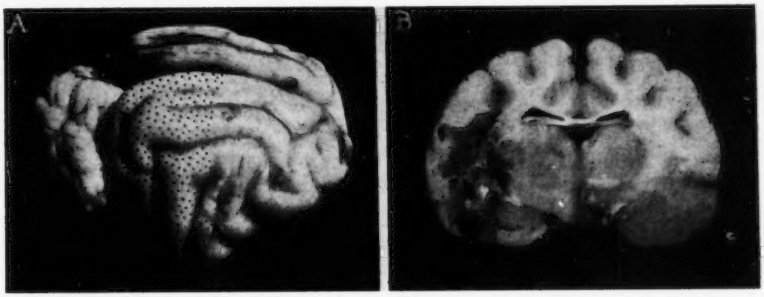

Fig. 4. Photographs of cat's brain from which the responses shown in figure 3 were obtained. A. Lateral view, indicating location of lesion. The stippled portion represents the area from which responses were abolished. B. Cross section, indicating extent of lesion.

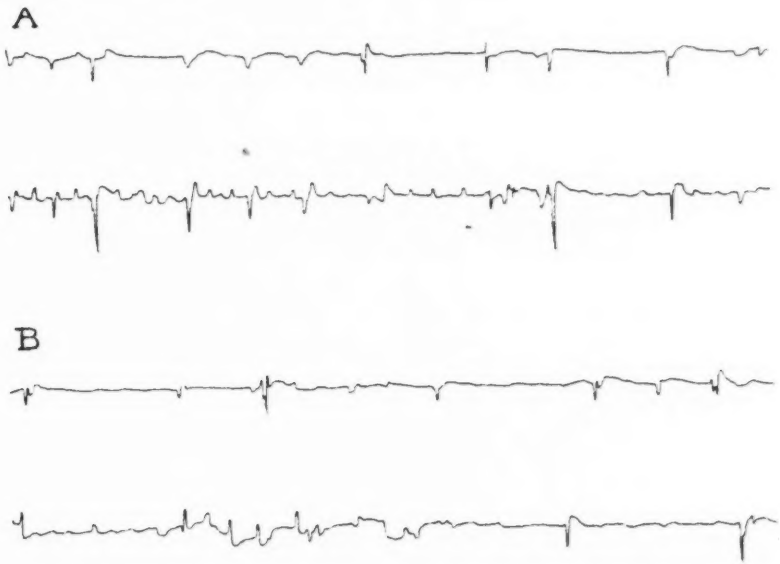

Fig. 5. Electrogram from an animal in which the left thalamus had been removed three weeks previously. Upper record, left posterior sigmoid gyrus; lower record, right posterior sigmoid gyrus. Paper speed, 15 mm. per sec. A. Record showing synchronization of spontaneous activity in the two hemispheres. B. Lack of synchronization after complete section of the corpus callosum.

A further more detailed analysis of these preparations is in progress, but as Dusser de Barenne and McCulloch (1938) have shown, interruption of thalamocortical circuits leads to permanent abnormalities of the cortical activity, especially noticeable as a suppression of the predominant cortical rhythm. The rhythmically occurring but widely separated waves may, on the other hand, actually be enhanced.

Throughout the course of the work tracing the cortical spread of the secondary discharge to sensory stimulation, it was noticed that lesions which abolished the latter also affected the spontaneously occurring waves of similar appearance. Callosal lesions which prevented the crossed secondary discharge dissociated the rhythmic activity of the two sides although spontaneous activity continued synchronously within each hemisphere. Temporal lobe lesions abolishing the secondary discharge in a part of either hemisphere also abolished the spontaneous waves of the same type. Midbrain lesions which abolished the secondary discharge (Dempsey et al., 1940) did not depress but in some preparations actually enhanced the spontaneous waves.

Discussion. It appears from the foregoing experiments that the secondary cortical discharge elicited by sciatic stimulation reaches the cortex over specific paths which arise deep in the temporal lobe in the region of the temporal horn of the ventricle and thence spread widely over the homolateral cortex. Furthermore, this region is in connection, via the anterior part of the callosum, with the corresponding contralateral area and is capable of activating through it the entire contralateral cortex. The spontaneous cortical rhythm made up of widely separated large waves similar in appearance to the secondary discharge may also be said to arise in this temporal area and to be synchronized throughout the cortex by means of the conducting system outlined for the secondary discharge.

It is impossible at present to give a specific localization to the active temporal area and even less satisfying to attempt a correlation of the present findings with what is known of the anatomy of these regions. Stimulation experiments (Morison, Dempsey and Morison, 1941) have shown that the response can be elicited from a region in which the most significant structure is the rostral pole of the amygdala. Several other basal telencephalic nuclei must be considered, however, and several notable fiber systems, of which the medial forebrain bundle, the stria terminalis, and the external capsule are the most prominent, might also have been involved in both the stimulation experiments and the lesions reported here. The external capsule would seem to offer the advantage of having the most generally admitted connections to the cortex, but evidence in the literature for extrathalamic somatic afferent connections for any of these areas is at best vague and unsatisfactory.

Another provocative finding is the connection of the two active basal

areas via a small portion of the callosum. It would certainly have been more in accord with known anatomy to find that the anterior commissure was responsible, but crossed responses were seen in several preparations in which it was completely divided. The relation of the corpus callosum to the older commissural systems has never been completely clear, however, and, of course, as old a structure as the hippocampus is intimately bound to the newer system. Could it not be therefore that some amygdala-amygdaloid or other baso-basal connections may have left the anterior commissure to travel with the callosum? It is hard to interpret the present results on any other basis.

It is difficult to suggest a correlation between this striking type of electrical activity of the cortex and the function of the cortex as it affects animal behavior. Secondary discharges similar to those under discussion have been elicited by various types of afferent stimulation, optic (Bishop and O'Leary, 1936) and even labyrinthine (Gerebetzoff, 1940). The latter author makes the intriguing suggestion that his responses may represent the generalized waking up of the cortex as the animal falls off a branch on which he is asleep. The notion has a good deal to recommend it and applies equally well to other sense modalities. In the present state of our knowledge, however, any behavioral correlates to cerebral action potential patterns are bound to be tentative.

SUMMARY

The presence in the cortex of a secondary response to sciatic stimulation homolateral to a hemisection of the brain stem was found to depend upon the presence of the anterior third of the corpus callosum. The opposite conjugate region of the cortex was not, however, essential (fig. 1).

Evidence is presented which indicates that in each hemisphere the response is distributed to all parts of the cortex by a fiber system which originates in an area close to the temporal horn of the ventricle (figs. 2 and 4). Other known cortico-cortical association systems are apparently incapable of spreading the response.

A type of spontaneous cortical rhythm is described which is dependent upon the same conducting system and not upon corticothalamic circuits (figs. 3 and 5).

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THE EFFECT OF DESICCATED HOG BILE AND HOG BILE ACID PREPARATIONS ON THE VOLUME AND CONSTITUENTS OF BILE[1]

A. L. BERMAN, E. SNAPP, A. C. IVY, V. H. HOUGH AND A. J. ATKINSON

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago

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The essential bile acid in hog bile is hyodesoxycholic acid, or 3-6 dihydroxycholanic acid. It is not found in human bile and differs from desoxycholic acid in that it has a hydroxyl group on carbon 6 instead of on carbon 7. The accepted chemical formula for hyodesoxycholic acid has been given in a previous publication (1). Recently, Fernholz (2) isolated from hog bile a keto acid whose formula was 3-hydroxy-6-ketocholanic acid. This was later confirmed by Schoenheimer et al. (3). According to Irvin, Mecker, Anderson and Johnston (4) hyodesoxycholic acid represents 88 per cent of the total bile acids found in dried fresh hog gall bladder bile; the remainder consists of 3-hydroxy-6-ketocholanic acid. These investigators also reported that 94 per cent of the hog bile acids are combined with glycine and approximately 6 per cent are combined with taurine.

Irwin, Johnston and Anderson (5) have administered desiccated hog bile orally to biliary fistula dogs and reported that it increased the volume output of bile and the total output of bile acids. The greatest increase occurred in the "desoxycholic acid fraction," which was obtained by the difference between the cholic acid and total bile acid output. No data were provided in their abstract and no comparison of desiccated hog bile with ox bile was mentioned.

Since we (1) have been interested in ascertaining the comparative response of the dog's liver to ox-bile salts and oxidized cholic acid salts, we have extended our studies to include hog bile salts.

METHODS. In this study the same methods[2] were used as those outlined in our previous paper (1).

Two preparations of desiccated hog bile were used, one a "purified"

[1] This work was aided by the E. L. Dawes and Marjorie Newman Research Grants.

[2] The carbonyl assays were made at the Wilson Laboratories, Chicago, by a method devised by Dr. E. L. Gustus of the Wilson Laboratories, Chicago.

preparation obtained from Wilson Laboratories, Chicago, and the other "Desicol" as marketed by Parke, Davis and Company. The Wilson product consisted almost entirely of alpha-glycohyodesoxycholic acid and contained 0 to 2 per cent carbonyl groups. Desicol contained 3.3 per cent carbonyl groups.

Oxidized desiccated hog bile was also used and contained 5 per cent carbonyl groups. In addition three different preparations of hyodesoxycholic acid were used. One was pure unconjugated hyodesoxycholic acid,

TABLE 1

Effect of hog bile salt preparations on the volume and constituents of bile

REGIME	NO. OF DOGS	NO. OF TESTS	VOLUME, CC./24 HRS.			CHOLIC ACID, MGM./24 HRS.			CHOLESTEROL, MGM./24 HRS.			PIGMENT, MGM./24 HRS.			TOTAL SOLIDS, MGM./24 HRS.		
			C*	T†	Per cent chg.	C	T	Per cent chg.	C	T	Per cent chg.	C	T	Per cent chg.	C	T	Per cent chg.
3 grams desiccated hog bile	5	10	176	242	+37	1442	1490	+3	13	13	0	177	160	−9	29	30	+3
3 grams Desicol	5	10	165	230	+39	1400	1510	+7	12	13	+8	171	173	+1	29	32	+10
3 grams oxidized desiccated hog bile	6	7	123	173	+40	1339	1280	−4	10	12	+20	89	82	−8			
3 grams unconjugated unoxidized hyodesoxycholic acid	5	8	171	237	+38	1394	1439	+3	16	14	−12	207	185	−10	29	29	0
3 grams oxidized unconjugated hyodesoxycholic acid	5	5	157	209	+33	1382	1365	−1	9	11	+22	108	117	+8	29	31	+7
3 grams oxidized conjugated hyodesoxycholic acid	5	6	153	198	+30	1440	1427	0	11	14	+27	195	168	−13	31	32	+3
3 grams ox-bile salts; 1.71 grams cholic acid.	8	15	133	182	+36	1493	2809	+88	12	16	+33	115	105	−8	36	44	+22
3 grams oxidized conjugated ox-bile salts	11	14	126	175	+39	1435	1559	+8	11	15	+36	104	100	−4	39	40	+2
3 grams Decholin dehydrocholic or oxidized cholic acid	6	10	128	264	+106	1473	1647	+12	11	8	−27	114	129	+13	38	30	−22
3 grams Ketochol or Kebilac, Ketocholanic acids	7	10	126	251	+99	1468	1568	+6	11	14	+27	114	105	−7	35	32	−9

* "C" = control.
† "T" = treated.

which contained 0 to 3 per cent carbonyl groups. Another was the oxidized unconjugated hyodesoxycholic acid, which contained 15.4 per cent carbonyl groups. The other was oxidized conjugated hyodesoxycholic acid, which contained 8.1 per cent carbonyl groups.

By studying these various types of desiccated hog bile and hog bile acids we should be able to ascertain the comparative effects of hog bile products on the secretion of bile and its constituents. Furthermore, since we believe that the hydrocholeresis produced by a bile acid may be asso-

ciated with the position and number of hydroxyl groups and also with the effect of oxidation and conjugation, a study of these 3,6-dihydroxycholanic derivatives should yield a greater knowledge of the possible mechanisms and causes of hydrocholeresis.

RESULTS. *Volume output of bile.* (Table 1.) Three grams of the two preparations of *desiccated hog bile* increased the volume output of bile by approximately 38 per cent. This amount of choleresis is very similar (36 per cent) to that obtained with 3 grams of "natural" ox-bile salts. That is, ox-bile salts and desiccated hog bile per gram weight are equal in increasing the volume output of bile.

Three grams of *oxidized desiccated hog bile*, which contained 5 per cent carbonyl groups and consisted almost entirely of oxidized glycohyodesoxycholic acid increased the volume output 40 per cent. This amount of choleresis is very similar (39 per cent) to that obtained with 3 grams of oxidized conjugated ox-bile acids. Thus, oxidation of the *conjugated* hog or ox-bile salts does not increase the choleretic action of the "natural" bile acids in hog or ox bile.

Three grams of *pure hyodesoxycholic acid* increased the volume output of bile by 38 per cent, which is the same as that obtained with desiccated hog bile and also oxidized conjugated hog bile, and also ox bile salts and oxidized conjugated ox bile salts (table 1).

Three grams of *oxidized hyodesoxycholic acid* increased the volume output by 33 per cent. This is most interesting since oxidation of cholic acid markedly increases its choleretic property, which is not true of hyodesoxycholic acid.

Three grams of *oxidized conjugated hyodesoxycholic acid* increased the volume output by 30 per cent, which is not quite as large an increase (40 per cent) as obtained with oxidized desiccated hog bile.

Comment. Thus, oxidation or conjugation of hyodesoxycholic acid does not significantly modify its choleretic property.

Cholic acid output. The normal variation in daily cholic acid output in these Rous-McMaster biliary fistula dogs was ± 10 per cent. Using this as our standard it can be seen from table 1 (cholic acid column) that desiccated hog bile and the various hog bile acid preparations did not change the synthesis or output of cholic acid. However, we have noticed that, when 3 grams of the hog bile preparations were administered, significant variations in cholic acid output occurred in the individual tests, the data for which are too bulky to present. For example, when 3 grams of the desiccated hog bile (Wilson) were given, in one case there was a decrease in cholic acid output of 19 per cent, and in another case an increase of 20 per cent. Variations in cholic acid output occurred with all the hog bile preparations that we used, but in all cases the decreases were relatively small and were counter-balanced by increases so that the average results

showed no appreciable change in cholic acid synthesis. In a previous report (1) we observed that occasional significant depressions in cholic acid output occurred when ketocholanic acids prepared from ox bile were administered, but only when large doses such as 5 grams were fed daily.

TABLE 2

Effect of hog bile salt preparations on the output of carbonyl groups in bile

BILE ACID	CC. PER DAY	MGM. TOTAL CHOLATES PER DAY	CONC. CHOLATES PER DAY	MGM. TOTAL KETO GROUPS PER DAY	CONC. KETO GROUPS PER CC.	TOTAL INCREASE DUE TO KETO ACID FED	MGM. EXCRETED OF KETO ACID FED	PER CENT RECOVERY OF KETO ACID FED
Desiccated hog bile (Wilson) 3 grams. 0-3% C=O group:								
Control	176	1442	8.1	53.0	0.30	0		
Treated	242	1490	6.1	52.8	0.21	0	0	
Desicol 3 grams. 3.3% C=O:								
Control	165	1400	8.4	53.0	0.32	0		
Treated	230	1510	6.5	42.9	0.18	−10.1	0	
Hyodesoxycholic acid 3 grams. 0-3% C=O group:								
Control	171	1394	8.1	53.0	0.31	0		
Treated	237	1439	6.0	37.6	0.15	−15.4	0	
Oxidized unconj. hyodesoxycholic 3 grams. 15.4% C=O:								
Control	157	1382	8.7	53.0	0.33	0		
Treated	209	1365	6.5	93.2	0.35	40.2	260	8.7
Oxidized conj. hyodesoxycholic 3 grams. 8.1% C=O:								
Control	153	1440	9.4	53.0	0.34	0		
Treated	198	1427	7.2	71.9	0.35	18.9	233	7.7
Oxidized hog bile 3 grams. 5.0% C=O:								
Control	123	1339	10.8	53.0	0.43	0		
Treated	173	1280	7.4	47.0	0.27	−6.0	0	

Output and recovery of carbonyl groups. Very little change occurred in the excretion of carbonyl groups when desiccated hog bile or hog bile acid preparations were administered orally (table 2). The total control output of carbonyl groups per day in this series of biliary fistula dogs was

53.0 mgm. Desiccated hog bile which only contained a small amount of carbonyl groups had no effect on the output of carbonyl groups. Desicol and pure hyodesoxycholic acid decreased the total output of carbonyl groups as well as the concentration per cubic centimeter. The latter decrease is only slightly significant. Both of these preparations contain approximately 3.0 per cent carbonyl groups. When oxidized conjugated hyodesoxycholic acid, containing 8.1 per cent carbonyl groups, was administered orally the carbonyl output was increased from 53 mgm. to 71.9 mgm. daily. This represented 233 mgm. of oxidized conjugated hyodesoxycholic acid excreted, or a recovery of approximately 8 per cent of the bile acid administered (1). In the calculation of the recovery of carbonyl groups, method III described in a previous paper was used (1). Oxidized unconjugated hyodesoxycholic acid which contains 15.4 per cent carbonyl groups increased the carbonyl output from 53.0 mgm. to 93.2 mgm. daily. The greater output of carbonyl groups is directly related to the greater amount of carbonyl groups present in this preparation. Approximately 260 mgm. of the oxidized unconjugated hyodesoxycholic acid was excreted which represents only a 9 per cent recovery. When the oxidized desiccated hog bile was administered, which contained 5.0 per cent carbonyl groups, there was no significant change in carbonyl output.

It can be seen that the oxidized hog bile preparations used did not greatly affect the carbonyl output in the bile. Approximately 8 per cent of the oxidized bile acids fed was recovered in the bile. This differs quantitatively from our observations on the metabolism of the oxidized ox-bile salts (1). When the oxidized natural bile acids were administered in 3 gram daily doses, from 16 per cent to 29 per cent was recovered during the actual period of administration. There was also a direct relationship between the amount of oxidized bile acid recovered in the bile and the percentage of carbonyl groups in the bile salt preparation that was administered. For instance, Dechacid no.14 contained 11.2 per cent carbonyl groups and the amount recovered was approximately 16.0 per cent (1). Ketochols which contained 18.3 per cent carbonyl groups, showed a 24.0 per cent recovery, while Decholin, containing 20.9 per cent carbonyl groups had a 28 per cent recovery. The oxidized hog bile acid preparations showed no such relationship. Although the percentage of carbonyl groups in the oxidized unconjugated hyodesoxycholic acid was twice as great as in the oxidized conjugated hyodesoxycholic acid, the amount recovered in either case was practically the same, approximately 8 per cent. What happened to the bulk of the hog bile acids administered is unknown. To be converted into cholic acid and excreted as such would require the addition of another hydroxyl group on carbon 12 and the transferring of the hydroxyl on C_6 to C_7. Such a transformation did not seem to occur since the cholic acid output in all cases was not increased over the control

output. The oxidized hyodesoxycholic acid preparations could have been reduced to the 3-hydroxy-6 ketocholanic acid or to hyodesoxycholic acid and excreted as such. Because of the lack of adequate quantitative methods for the determination of these hog bile acids in dog bile, further information as to the fate and recovery of these hog bile acids was not obtained. As indicated by the observations of Irvin et al. (5), the hyodesoxycholic acid probably was excreted in the undetermined bile acid fraction.

Effect of cholesterol output. All of the hog bile acid preparations except the unoxidized unconjugated hyodesoxycholic acid caused an increase in cholesterol output. However, in no case was the increase physiologically significant although 3 grams of oxidized conjugated hyodesoxycholic acid gave a significant statistical increase in cholesterol output. When the pure hyodesoxycholic acid was administered in 3 gram daily doses there occurred a 12 per cent decrease in cholesterol output. This result is not significant, physiologically or statistically. None of the eight tests on five dogs showed a marked decrease in cholesterol output as we observed when 3 and 5 grams of Decholin were administered (1).

Effect on pigment output and total solids. The hog bile salt preparations studied had no significant effect on pigment output. Thus, neither the hog bile acids nor any of the bile acids studied by us, whether oxidized or unoxidized, materially affected the output of bile pigment.

None of the hog bile acids studied affected the concentration of total solids significantly. From the standpoint of non-volatile solids, the desiccated hog bile and the hog bile acid preparations did not "thicken" the bile as do the "natural" conjugated ox-bile salts, nor "thin" the bile as do the oxidized ox-bile salts (Decholin, Ketochol and Kebilac).

Discussion. This work represents an extension of our studies on bile acid metabolism in which we desire to ascertain the effect of various bile acids on the secretion of bile, the relationship of the structure of the bile acid to its choleretic effect, and finally, to study the action and the metabolism of the oxidized bile acids. In a previous study (1) we found that the oxidized unconjugated acids, such as oxidized cholic acid (Decholin) and the mixture of oxidized cholic, desoxycholic and lithocholic acids (Ketochol and Kebilac) were the most potent choloretics per gram weight. These preparations consist primarily of the tri-ketocholanic acid or oxidized cholic acid with the carbonyl groups on C_3, C_7, and C_{12}. It is interesting that hyodesoxycholic acid with the hydroxy groups on C_3 and C_6, whether it is conjugated or not, yields the same amount of choleresis as unoxidized conjugated and oxidized conjugated ox-bile acids. Since the oxidation of cholic acid increases its choleretic property and since the oxidation of conjugated cholic acid does not increase its choleretic property, conjugation might be presumed to decrease the choleretic effect of oxidation.

This may be true of cholic acid, but it certainly is not true for hyodesoxycholic acid, since oxidation of hyodesoxycholic acid or of its conjugated derivative glycohyodesoxycholic acid does not augment its choleretic action. It appears that the position of the carbonyl group or groups in the cholane nucleus is important in determining the choleretic property.

The desiccated hog bile and the hog bile acid preparations have no appreciable effects on the constituents of bile such as cholic acid, cholesterol, pigment and total solids. Hyodesoxycholic, which is the essential bile acid in these preparations, seems to be handled differently by the liver than any of the other bile acid preparations that we have studied. Relatively little of the carbonyl groups administered in these bile salts can be recovered in the bile. It is quite possible that the liver has changed the oxidized hyodesoxycholic acid to such an extent that our present chemical methods will not detect it in the bile. Adequate quantitative chemical methods are needed for the detection and the determination of hyodesoxycholic acid and its related keto acid before a complete knowledge of the metabolism of hyodesoxycholic acid can be elucidated.

Thus, when hog bile preparations are used in man, as judged from our results on dogs, they will produce an increase in bile volume output equivalent to that obtained with equivalent amounts of unoxidized conjugated ox-bile salts or "natural" ox-bile salts. They will not in equivalent amounts cause as much choleresis as oxidized unconjugated cholic acid (ox-bile). The hog bile preparations do not "thin" the bile like oxidized unconjugated cholic acid (Decholin, Ketochol and Kebilac). When hog bile preparations are used, hyodesoxycholic acid, a bile acid apparently foreign to human bile is excreted into the intestine. This is not true when ox bile salts or oxidized cholic acid preparations are used. Whether this difference is of physiologic significance is unknown and moot.

SUMMARY AND CONCLUSIONS

1. Three grams daily of desiccated hog bile caused a 38 per cent (average) increase in the output of bile in biliary fistula dogs. This is the same as the increase obtained with 3 grams of ox-bile salts containing from 1.5 to 1.7 grams of cholic acid.

2. Three grams of pure hyodesoxycholic acid, the chief bile acid of hog bile, increased the output of bile to the same extent (40 per cent) as the desiccated hog bile.

3. Oxidation of desiccated hog bile or of the pure hyodesoxycholic acid did not augment the choleretic property of these bile acids. This is in contrast to the results obtained with cholic acid, the chief bile acid in ox bile.

4. Conjugation of oxidized hyodesoxycholic acid had no effect on the choleretic property. All the preparations of hog bile had about the same choleretic activity.

5. Whether oxidation of a bile acid augments its choleretic activity depends on the position of the carbonyl groups, and whether the conjugation of an oxidized bile acid decreases its choleretic activity depends on the position of the carbonyl groups.

6. The doses of the hog bile preparations used did not significantly modify cholic acid output, though the output of cholic acid was rendered more variable.

7. The hog bile preparations tended to increase total cholesterol output slightly, but had no significant effect on the total output of pigment and the concentration of non-volatile solids. The bile is not "thinned" by hog bile preparations as it is with oxidized unconjugated cholic acid preparations made from ox bile.

8. The recovery of material containing carbonyl groups in the bile is even less when oxidized hyodesoxycholic acid is given than when oxidized cholic acid is given (1).

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THE RELATION BETWEEN ELECTRICAL AND MECHANICAL EVENTS IN THE DOG'S HEART[1]

J. A. E. EYSTER, WALTER J. MEEK AND HAROLD GOLDBERG

From the Physiological Laboratory, University of Wisconsin, Madison

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In a preceding publication (1) concerned with the relations between electrical and mechanical events in the turtle's heart, the following facts were established: *a*. The onset of local contraction of surface muscle of the ventricles is coincident or nearly coincident in all regions, with the main peak of the differential potential-time curve derived from the region. *b*. Peaks on the differential curve occur during gradients on the unipolar potential-time curve and are proportional to the size of the gradient. The present communication is concerned with an extension of this study to the dog's heart.

METHODS. Myograms were recorded from local surface regions of the ventricles by means of an electrical resistance myograph, similar to the one used in the studies on the turtle (1). Local shortening of muscle segments results in an increase in the flow of electrical current through the myograph and the changes in current flow are amplified and recorded by a cathode ray oscilloscope. In one series of experiments myograms were recorded simultaneously with differential potential-time curves from the same region. To record the latter, the two arms of the myograph were provided with wick electrodes leading to a second amplifier and oscilloscope. In another series of experiments, the relations between myograph and pressure curves from the two ventricles and from the pulmonary artery were studied by recording the two curves simultaneously. For recording intraventricular or arterial pressures, a membrane manometer with photo-electric recording was used as in the previously reported experiments on the turtle's heart (2). In a third series of experiments, differential and unipolar potential-time curves were recorded simultaneously from various regions on the surface of the ventricles and the right auricle. Two zinc zinc-sulphate electrodes provided with a common wick, were mounted close together. The middle of the wick made contact with a small area of the cardiac surface and was held in place by a lightly stretched thread. Leads from the two electrodes to one amplifier and oscilloscope provided for the differential recording. Leads from one of the electrodes

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and from a distal electrode on a hind leg to a second amplifier and oscilloscope provided for the unipolar recording.

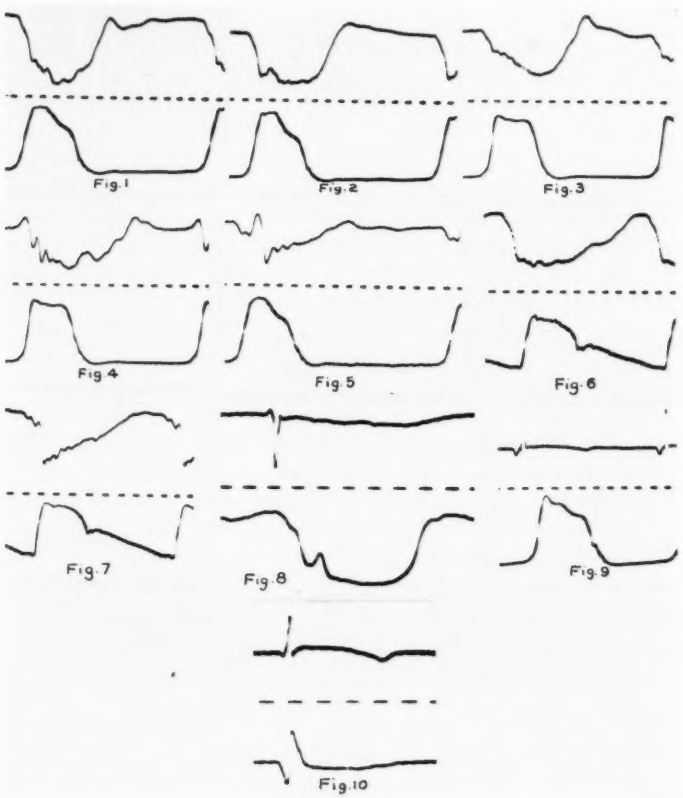
All experiments were carried out on large or medium sized dogs under morphine-ether anesthesia. The hearts were exposed in situ with open pericardium. The work was done in a laboratory surrounded by a double shield of wire netting to avoid interference from electrical strays. Measurements of all records were made with a micrometer comparator with 40X magnification.

RESULTS. *The relation of myograph and pressure curves.* In previous work on the turtle's ventricle (2), a clear relation was found to exist at all surface points between the onset of local contraction and the rise of intraventricular pressure. The intraventricular pressure curve shows an initial slow rise of pressure of a few millimeters of mercury, lasting about 0.12 sec. and terminating in a sharp increase in the pressure gradient. It was found that the onset of contraction of all surface points occurred during this initial rise of pressure or followed it by a brief interval. The occurrence of actual shortening at all parts of the ventricle during the isometric period was explained by the change of shape of the ventricle to approximate a sphere which occurs early in ventricular systole. No regions were found in the normally beating heart where the myograms indicated an initial dilatation.

The intraventricular pressure of the dog shows a similar initial slow rise followed by a sudden increase of the pressure gradient. The initial period however lasts only about 0.02 sec. and the pressure rise during it is somewhat greater than in the turtle. Myograph curves recorded from certain regions of the right and left ventricles of the dog show a close resemblance to those recorded from the ventricle of the turtle. The onset of contraction is sharply defined and occurs at various times during the initial period of slow rise of intraventricular pressure. Examples of curves of this type are given in figures 1, 2 and 3. The upper curve is the myogram, shortening of the local region of muscle being indicated by a downstroke. In figures 1 and 2 the myograph was on the right ventricle and the lower curve records pressure in the right ventricle, a rise of pressure being indicated by an upward movement. In figure 1, local contraction is recorded from a region of the ventricle contiguous to the interventricular groove. The onset of contraction is coincident with the first detectable rise of intraventricular pressure; i.e., with the start of the initial period of pressure rise. The myograph in figure 2 was on the mid-anterior surface of the right ventricle in the same heart. The onset of contraction follows the onset of the initial pressure rise by 0.0112 sec. and precedes the end of this period by 0.0053 sec. Figure 3 is a myogram from a region on the left ventricle near the groove and a record of pressure in the left ventricle. The onset of contraction follows the onset of the initial rise of pressure by

0.0076 sec. and precedes the occurrence of the sharp increase in pressure gradient by 0.0124 sec. In all figures the middle line of dashes represents time intervals of 0.04 sec.

Myograms of the type described above are the rule in records obtained from near the interventricular groove, particularly on the right ventricular side. There is experimental evidence to indicate that these are the earliest



Figs. 1–10

surface regions of the two ventricles to enter into contraction and would be expected to be least influenced by local contraction of muscle occurring elsewhere. Other regions, as shown in figure 2, may however exhibit the same clear onset of local contraction.

In certain myograms, however, recorded from the surface of the ventricles of the dog, in contrast to those from the ventricle of the turtle, the

onset of local shortening is not clearly defined. The region may show a dilatation lasting throughout the isometric period of the ventricle as shown in figure 4. These myograms resemble those recorded by Tennant and Wiggers (3) in a previous study. At still other regions, the onset of shortening may be indicated, but the shortening is quickly replaced by a dilatation of the region until after the isometric period is completed (fig. 5).

The relation of the onset of shortening of various regions to the isometric period of the ventricle is shown by records in which myograms are recorded from the surface of the right ventricle along with pressure from the pulmonary artery. At regions near the interventricular groove, local shortening may reach almost its maximum before the end of the isometric period of the ventricle, as indicated by the rise of pressure in the pulmonary artery. In figure 6, the myogram is from a region on the anterior surface of the right ventricle contiguous with the groove. The onset of shortening occurs 0.0558 sec. before the start of the rise of intrapulmonic pressure. The end of the rapid period of shortening and the end of the isometric period are coincident. At other regions, particularly those along the base of the ventricle and near the conus, shortening may be in large part delayed until the end of the isometric period. In figure 7, the myogram is from a region near the conus. The onset of rapid shortening in this region does not occur until about 0.01 sec. after the end of the isometric period, and the occurrence of full shortening is coincident with the maximum pressure in the pulmonary artery.

It would hence appear clear that at certain local regions of the surface of the ventricles of the dog, shortening of the muscle occurs unimpeded by the rise of intraventricular pressure, and bears the same time relation to the intraventricular pressure as in the turtle. At other regions, however, contrary to the situation in the turtle, shortening is either more or less impeded by the rise of intraventricular pressure resulting from contraction of ventricular muscle in other regions, or the muscle itself has a slower rate of contraction.

The relation of the peak of the differential curve to the onset of shortening. Simultaneous recordings of myograms and differential potential curves from regions on the surface of the ventricles of the dog show that at certain regions at least, the same relation exists between the onset of shortening and the main peak of the differential curve as we have previously found in the turtle. In regions from which myograms can be obtained which indicate clearly the onset of shortening, the start of this process is synchronous or nearly so with the main peak of the differential curve. An example is given in figure 8. The upper curve records the potential at the differential electrode. The lower curve is the myogram from the same region. The records were made from a region on the right ventricle close to the groove. The onset of shortening is synchronous with the main

peak of the differential curve. In 30 similar records, obtained from 5 experiments, the onset of shortening and the main peak of the differential curve were synchronous in 16. In 10, the main differential peak appeared to precede and in 4 to follow the onset of shortening by intervals of less than 2 milliseconds. As an average of all the records, shortening followed the main differential peak by 0.4 millisecond, a figure which is probably not significant.[2]

As a corollary to the relation of the myograph and differential curves, it is found that the main peak of the differential curve has the same relation to the intraventricular pressure as does the onset of local shortening to this pressure. Simultaneous recordings of differential potentials and intraventricular pressure show that the main peak of the differential curve derived from various surface regions falls within the initial period of slow rise of pressure. An example is given in figure 9. The differential potential-time curve is from a region on the right ventricle a short distance above the groove. The main peak of the differential curve occurs 0.0093 sec. after the start of the initial period of slow rise of pressure in the right ventricle, and 0.0129 before the end of this period. At certain regions contiguous to the groove, the main peak of the differential curve and the start of the rise of intraventricular pressure may be simultaneous.

It is thus possible to show that at certain regions the same fundamental relation exists between the mechanical event of shortening and the electrical state, as expressed by the main peak of the differential potential-time curve on the surface of the dog's ventricles, as may be demonstrated at all regions on the ventricle of the turtle. It has not been possible to demonstrate this relation at all regions on the surface of the ventricle of the dog because at certain regions myograms fail, as has been discussed in the previous section, to reveal the instant at which the local region of muscle enters into the shortening process.

Relation of unipolar and differential potential time curves. From most regions of the surfaces of the right auricle and ventricle, the unipolar curve is diphasic, with the first peak in the positive direction. The interval between the two peaks in the dog is of the order of 0.01 sec. or less, as

[2] In order to estimate the significance of differences in time relations of curves measured under the conditions of the present work, a record was made of simultaneous recording of differential and unipolar potential curves from a heart in which 50 successive cycles were recorded. The record was made at a recording speed such that one millimeter along the time axis corresponded to 9.0 milliseconds. Measurements were made between a sharp peak on each curve in the 50 cycles and submitted to statistical analysis. The principal results, expressed in milliseconds, are as follows. Mean, 9.18 ± 0.047. Standard deviation, ±0.33, ±0.033. Coefficient of variation, 3.8 per cent. Three times the standard deviation is approximately one millisecond. This degree of variation corresponds to a difference of about 0.11 mm. on the record.

compared to an interval of about 0.04 sec. in the turtle. It is in this interval that the maximum time gradient, i.e., the most rapid rate of change of potential occurs in all but a few cases. Occasionally the most rapid time gradient occurs on the distal limb of the negative peak or on the proximal limb of the positive peak. At a few regions of the right auricle and ventricles, the potential grows to a positive or negative value and maintains this polarity throughout the QRS period. Notably is this true over the upper part of the sulcus terminalis of the right auricle. As previously reported by Wilson, Macleod and Barker (4), this region gives unipolar curves which are monophasic in the negative direction. In monophasic unipolar curves the growth of the potential is rapid, the decline slow, the maximum gradient occurring on the proximal limb.

The differential curve from the dog's heart shows consistently the same relation to the unipolar curve that we have previously reported for the tortoise heart; the main peak of the differential curve occurs coincidentally with the period of maximum gradient on the unipolar curve. Differential peaks occur during gradients of the unipolar curve, and their magnitudes are proportional to these gradients. An example of the usual type of curve is given in figure 10. Small downwardly directed peaks occur on the differential curve coincident with the small gradients associated with the development of the initial positive potential and with the decline of the final negative potential of the unipolar curve. The maximum peak on the differential curve falls approximately midway between the positive and negative peaks of the unipolar curve during the period of most rapid change of potential.

Discussion. That different regions of the right auricle of the dog's heart start shortening at different time instants has been shown by C. J. Wiggers (5) and by Lewis, Feil and Rothschild (6). The former investigator used a small mechanical myograph with optical recording and developed the conception of *fractionate contraction* of different regions as contrasted with the contraction of the organ as a whole as recorded by the usual suspension methods. Lewis, Feil and Rothschild worked with a grid of threads attached at one end to the auricular wall. Local contractions were indicated by approximation of adjacent threads as determined by photographic recording. That fractionate contraction characterizes the mechanical activity of the ventricles also, is evident from the experiments reported in the present communication.

With reference to the relation which exists between the onset of fractionate contractions, intraventricular pressure and the electrical state in the region, the following statements may be made. From experiments on turtle hearts, reported previously, and from the experiments on dog hearts, reported in the present communication, two important relations have been established for all surface regions of the ventricles of the turtle and the dog.

These are, first, that the main peak of the differential potential-time curve from all surface regions occurs during the period of initial slow rise of intraventricular pressure and second, that peaks on this curve coincide with gradients on the unipolar curve recorded from the same region and are of a magnitude proportional to the magnitude of the gradient. It has been further established, for all surface regions of the turtle's ventricle and from many but not all regions of the dog's ventricles, that the main peak of the differential curve is coincident or nearly coincident with the onset of fractionate contraction and that the onset of fractionate contractions from the various surface regions falls within the initial period of slow rise of intraventricular pressure. The inability to demonstrate these relations for all surface regions on the dog's ventricle is due, as stated above, to interference with the onset of the shortening process in certain regions. No exceptions from the relations noted have been found in any region from which satisfactory curves of shortening have been obtained.

The experimental results, we believe, clearly justify the conclusion that, in general, there is a close relation existing in cardiac muscle between the onset of the contraction process in any region and the presence of an electrical state which is defined by the differential potential-time curve recorded from this region. The maximum peak of this curve which is coincident or nearly coincident with the onset of contraction, signals the maximum flow of electric current and the maximum time rate of change of current[3] in the region from which it is derived, established by a potential gradient existing between neighboring regions at which the potentials are respectively above and below the potential of the resting muscle. This instant is also characterized by the most rapid time rate of change of potential in the region, as shown by the unipolar potential-time curve.

CONCLUSIONS

Different local regions of the surface of the ventricles of the dog's heart start shortening at different time instants. The occurrence of mechanical activity, as indicated by the onset of the fractionate contraction in any region, is coincident with or separated by a brief interval from the occurrence of maximum flow of electric current and maximum time rate of change of current, resulting from a potential gradient established between neighboring regions in which the potential is respectively above and below the potential of resting muscle. This instant is signalled by the occurrence of the main peak of the differential potential-time curve and by the occurrence of the maximum gradient on the unipolar potential-time curve derived from the region that is entering into activity.

[3] It is possible that the rapid time rate of change of current associated with the large gradients immediately preceding and following the apex of the peak, may be interrupted for a brief interval. This interval, if it exists, must be so small, however, that it has no practical significance in the present work.

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A COMPARISON OF THE HISTAMINE CONTENT OF BLOOD AND BONE MARROW[1]

CHARLES F. CODE AND JAMES L. JENSEN

From the Department of Physiology, University of Minnesota, Minneapolis

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It has been shown that the white cell layer of centrifuged unclotted blood from normal animals contains most of the histamine found in blood (Code, 1936; Bram Rose, 1939). Further study has indicated that, of the cells comprising the white cell layer, those originating in the bone marrow are richest in histamine (Code, 1937a; Zon, Ceder and Crigler, 1939). In this investigation an attempt has been made to determine whether or not the cells of the blood contain the histamine when they leave the bone marrow by comparing the histamine content of the blood with that of the bone marrow. If the bone marrow were free of histamine it might be concluded that the cells obtained their histamine after leaving the marrow.

METHODS. As a routine, animals were killed instantly by a blow or a shot to the head. Blood was taken before or immediately afterward from the heart. In some instances ether or nembutal anesthesia was used. The two femora were removed, split or partly sawed and then split in two and samples of marrow taken and weighed. The entire operation was carried out without interruption. In order to detect any abnormality of the white blood cells, white cell counts were made of the blood and smears of blood and imprints of marrow were taken for examination. The smears and imprints were stained with Wright's stain. In all differential white cell counts of the blood smears at least 300 cells were counted. In most instances the lymphocytes and monocytes were not recorded separately. In the course of this investigation blood and bone marrow were taken from guinea pigs, rabbits, cats, dogs, one horse and one cow.

A procedure (Code, 1937b) modified after the original Barsoum and Gaddum (1935) method was used for the estimation of histamine in both blood and bone marrow. Duplicate samples of 5 cc. blood were taken for extraction except in one instance with the guinea pig when insufficient blood was obtained for duplicate estimations. In the guinea pig, rabbit

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and cat the entire femoral bone marrow was easily shelled out as a solid red clot which assured a uniform sample from each bone. Because of the small quantity of marrow obtained from the guinea pig the samples for the two femora were weighed and extracted together, but in the case of the rabbit and cat they were often extracted separately. With the dog and the large domestic animals, uniform samples containing red marrow were not obtained because of the spotty distribution of the islets of red tissue and the difficulty in removing the marrow from the bony spicules. Yellow marrow which to the naked eye was free of red tissue was obtained in abundance from the horse and cow.

When extracts were prepared from bone marrow certain additions to the usual procedure were necessary. In order to insure uniform extraction and complete protein precipitation, the marrow was thoroughly ground with clean sand and a considerable excess of trichloracetic acid slowly added. Droplets of fat could often be seen in the trichloracetic acid filtrates of yellow marrow. The excess fat was removed by shaking once with ether.

In some instances, as a check upon the nature of the substance estimated, final water and alcoholic extraction of both the blood and bone marrow was made for comparison. It has been found that the recovery of histamine added to blood is often low when final alcoholic extraction is used (Code, 1937b). To insure more complete recovery the procedure indicated by Anrep, Barsoum, Talaat and Wieninger (1939) of frequently repeated extractions with relatively large amounts of alcohol has been followed. The amount of alcohol used in the present study for extraction per unit volume of blood was twice or more than twice that advised by Barsoum and Gaddum.

All histamine assays were made using the lower ileum of the guinea pig suspended in Tyrode' solution containing atropine sulphate. The estimations are given in terms of gamma histamine base. The quantity of blood used for extraction was measured by volume but in order to make the determinations more comparable with those of the bone marrow the blood values have been computed to gamma per gram by using an average specific gravity value for blood of 1.050 (Barbour and Hamilton, 1924; Nice and Katz, 1935).

Results. *Guinea pig.* In the guinea pig the bone marrow contained from 27 to over 170 times as much histamine as the blood. The bone marrow histamine ranged from 10.4 to 22.2 γ per gram with an average concentration of 15.2 γ per gram (table 1). With the exception of one animal the histamine content of the blood ranged from 0.225 to 0.067 γ per cubic centimeter. These values are within the maximal and minimal concentrations of 0.280 and 0.066 γ per cubic centimeter noted in an earlier study of the blood of 20 normal guinea pigs (Code, 1939). The exception

TABLE 1

A comparison of the histamine content of the blood and bone marrow in the guinea pig, rabbit and cat

ANIMAL	NO.	WHITE BLOOD CELL COUNT					BLOOD HISTAMINE		MARROW HISTAMINE	M.H./B.H.
		Total	Differential per cent							
			N	E	B	L & M				
		thousands					γ/cc.	γ/gram	γ/gram	
Guinea pig	1	10.6	51.0	3.3	0.7	45.0	0.067	0.064	10.4	162
	2	8.0	30.0	9.0	0.3	60.7	0.167	0.159	22.2	140
	3	15.5	39.0	4.7	0.7	55.6	0.225	0.214	12.5	58
	4	14.3	31.7	2.7	0.0	65.6	0.069	0.066	11.8	179
	5	10.1	57.2	0.7	0.7	41.4	0.871	0.830	22.2	27
	6						0.160	0.152	15.0	99
	7						0.178	0.170	12.0	71
Average									15.2	
Rabbit	1	7.8	19.0	1.2	3.2	76.6	2.22	2.12	10.00	4.7
	2	8.8	22.7	0.3	1.7	75.3	2.00	1.91	6.66	3.5
	3	7.5	42.3	1.3	0.4	56.0	1.95	1.86	8.42	4.5
	4	7.7	39.3	0.4	2.3	58.0	2.50	2.38	11.05	4.6
Average									9.03	
Cat	1	11.3	42.0	5.0	0.0	53.0	0.044	0.042	3.05	76
							0.044†		3.37	
							0.044*			
	2	15.6	47.3	4.0	0.3	46.4	0.047	0.044	13.12	296
							0.045		12.90*	
	3	14.6	53.0	6.0	0.0	41.0	0.053	0.052	4.00	77
							0.057*			
Average									6.74	
	4	28.4	37.0	2.0	0.3	60.7	0.436	0.405	30.77	77
							0.414*		32.00	

Explanatory notes. The percentage of neutrophils, eosinophils, basophils and lymphocytes and monocytes found in the differential count is given under the columns N, E, B and L and M respectively. The histamine concentration in the duplicate samples of blood and marrow are shown for the cat while in the case of the other animals the duplicates were averaged to give the values tabulated. The figures in the last column were obtained by dividing the blood histamine (B.H.) into the marrow histamine (M.H.). The blood sample marked † was ground with sand in order to test the effect of this procedure on the histamine estimation. Final alcoholic extraction was used in the samples marked with an asterisk.

(animal 5, table 1) had a blood histamine of 0.871 γ per cubic centimeter. None of the observations made offered an explanation for this abnormally high value.

The rabbit. In the four rabbits tested the bone marrow contained from 3.5 to 4.6 times as much histamine as the blood. The bone marrow histamine content was between 6.66 and 11.05 γ per gram with an average concentration of 9.03 γ per gram (table 1). The white cell counts and blood histamine values were within normal values.

The cat. In four cats the histamine content of the bone marrow was consistently higher than that of the blood (table 1). The bone marrow of three of those animals was almost 76 times richer in histamine than the blood, while in one animal the ratio was 296 (cat 2, table 1). In one animal there was a leucocytosis of 28,350. The remaining three normal cats gave blood histamine values between 0.04 and 0.06 γ per cubic centimeter and bone marrow concentrations of 3 to 13 γ per gram with an average of 6.74 γ per gram. The blood of the animal showing the leucocytosis contained 0.4 γ histamine per cubic centimeter, or about 10 times as much histamine as the normal members of the group. The concentration of histamine in the bone marrow was more than twice the highest value obtained in the normal animals. This finding emphasizes the importance of a careful examination of blood when determining normal blood histamine values.

The dog. The histamine content of the bone marrow of the dog always exceeded that of the blood (table 2). The bone marrow contained 0.07 y to 0.96 γ per gram and in most instances, as is usual in the dog, the blood was free of histamine. The dog's bone marrow histamine content was considerably lower than that of the guinea pig, rabbit or cat. This difference in histamine concentration was associated with a distinct difference in the character of bone marrow in these animals. In the guinea pig, rabbit and cat the bone marrow had the appearance of homogeneous red clot which could be shelled out of the bone without great difficulty. In the dog the marrow was composed of a mixture of red, cellular material and yellow fatty substance which was closely adherent to the bony spicules and could not be removed in a block. In two instances in the dog an attempt was made to separate the red from the yellow marrow. The separation was not complete. The samples obtained were, at best, either predominantly red or yellow and for that reason were referred to as reddish or yellowish marrow respectively. The reddish marrow contained more histamine than the yellowish marrow (table 2).

Horse and cow. Because the yellowish marrow of the dog contained some histamine it seemed possible that the tissue forming the framework of the marrow contributed to the histamine content of the whole marrow. Pure yellow marrow was obtained from the femora of a horse and a cow.

One sample of yellow marrow from the horse contained a quantity of histamine which could just be detected. All other samples of yellow marrow from the horse and cow were free of histamine (table 2). In contrast to the yellow marrow, the red marrow obtained from these animals contained significant quantities of histamine. The greater proportion of red

TABLE 2

A comparison of the histamine content of the blood and bone marrow in the dog, horse and cow

ANIMAL	NO.	BLOOD HISTAMINE		MARROW HISTAMINE, γ/GRAM		
				Reddish	Yellowish	Yellow
		γ/cc.	γ/gram			
Dog	1	0.018	0.013	0.958	0.781	
		0.010				
	2	0.00	0.00	0.500	0.215	
		0.00			0.215*	
	3	0.00	0.00		0.080	
		0.00			0.070	
	4	0.00	0.00	0.400	0.277	
		0.00		0.384*	0.200*	
		0.00*				
		0.00*				
Horse	1	0.027	0.029	2.00		0.017
		0.033		2.06		0.00
Cow	1	0.015	0.014	0.027		0.00
		0.015				0.00
		0.015				

Explanatory notes. The histamine values for the duplicate and triplicate samples of blood and bone marrow are recorded. Activity less than 0.01 y per cc. or gram was regarded below the limit of accurate histamine estimation and recorded as 0.00 γ. Most samples so indicated were actually free of any recognizable histamine activity. Final alcoholic extraction was used in the samples marked with an asterisk. The white blood cell counts for the dogs were normal. The blood cells were not counted in the horse and cow.

tissue found in the femur of the horse was associated with a higher concentration of histamine than that found in the reddish marrow of the cow (table 2).

Final alcoholic extraction. In the final stages of the preparation of the extracts of blood and bone marrow either water or alcohol was used to extract the dried residue obtained after acid boiling. As a routine the

extraction was made with water but in three experiments in the cat and one in the dog extracts were prepared with alcohol (tables 1 and 2). In these instances water extraction was carried out on identical samples for comparison. In order that differences in grinding the marrow with sand and trichloracetic acid might not affect the results, the marrow was treated as one batch and then the trichloracetic acid filtrate divided into equal samples, one for extraction with water, the other for treatment with alcohol.

In one instance, the histamine content of the alcoholic extract of the blood was slightly above that prepared with water although the difference was not significant (cat 3, table 1). In all other cases, the alcoholic extracts contained the same or somewhat less histamine than the water extracts. Alcoholic extractions of the yellowish marrow in one experiment gave a histamine concentration of 0.200 γ per gram while extraction with water yielded 0.277 γ histamine per gram. With this exception, agreement between the alcohol and water extracts was sufficient to allow the conclusion that the active substance estimated was the same in the two extracts.

Discussion. In all animals tested the histamine content of the bone marrow was greater than that of the blood. The whole blood histamine concentration however may not be the best basis for comparison since it includes the plasma which is poor or lacking in histamine and not present to the same extent in the marrow. The plasma of the blood of the animals used in this study is seldom more than two-thirds of the whole blood. When this ratio is used to correct the histamine content of the blood to represent gamma histamine per gram of cells the marrow still contains more histamine than the blood, although in the rabbit the difference is hardly significant.

The histamine content of the red marrow obtained from the guinea pig, rabbit and cat was greater than that of the mixed yellow and red marrow found in the dog, horse and cow. In the horse and cow the red cellular marrow contained the histamine while the inactive fatty marrow and its supporting frame work were free or practically free of histamine. The results indicate that the portion of the marrow most closely concerned with the production of blood cells contains the histamine.

Since the white cell layer of centrifuged normal blood contains most of the histamine found in blood, it seems likely that the histamine in red marrow is associated more with the white cell than with the red cell producing elements. This contention receives support from the finding that in myelogenous leukemia when immature white cells are present in the blood stream the blood histamine is elevated (Marcou, Parhon and Comsa, 1936; Code and Macdonald, 1937).

The constituents of the white cell layer of centrifuged blood arising in

the bone marrow are the granular or myeloid leucocytes and the platelets. Both types of cells may contain histamine. In most animals accumulated evidence indicates the myeloid leucocytes as the cells containing histamine in the blood. In the rabbit, however, unlike man and the horse, platelet deposits obtained by differential centrifugalization of the blood may be rich in histamine (Code, 1937a). Further, Zon, Ceder and Crigler (1939) have found a drop in the blood histamine of rabbits when the platelet material of the blood was reduced by the administration of antiplatelet serum. In the rabbit it seems possible that the property of the myeloid leucocytes to carry histamine is shared by the other constituent of the white cell layer which arises in the bone marrow, namely, the platelet.

The alcohol-insoluble, water-soluble active factor found by Anrep, Barsoum, Talaat and Wieninger (1939) in the red cells of dogs in Egypt was not encountered in this study. The good agreement between the histamine concentration of alcoholic and water extracts of similar samples of blood and marrow indicated only histamine was being estimated. It seems unlikely that the alcohol-insoluble, water-soluble factor found in Egypt has even played a significant rôle in the histamine studies carried out with the modified method in England and the United States. During the past four years red cells of dogs in England and the United States have been frequently tested using a final water extraction and in nearly all instances the extracts have been found to be free of histamine, indeed free of a significant quantity of any substance which would contract the guinea pig ileum (Code, 1937a, 1939).

The satisfactory yield of histamine when final alcoholic extraction was used in this study was believed due to prolonged extraction with excess alcohol. Using alcoholic extraction as directed in the original Barsoum and Gaddum method (1935), Code (1937b) obtained unsatisfactory agreement between duplicate extracts of 10 cc. of blood in about one-third of the samples and noticed a failure of the alcoholic extraction of this method to yield a consistently satisfactory recovery of histamine added to blood. The difficulty of obtaining a sufficient recovery with alcohol seems partly mechanical. In the isolation of histamine from white blood cells Code and Ing (1937) used alcohol in eight instances to extract different batches of the dried residue obtained from trichloracetic acid filtrates. The residue was generally a solid cake on the side of the flasks and this had to be broken and subjected to repeated, prolonged extraction to give complete recovery of histamine. In the routine preparation of extracts of blood the residue obtained after drying the boiled trichloracetic acid filtrates often forms a thin hard layer on the flask the bulk of which is insoluble in alcohol. Anrep, Barsoum, Talaat and Wieninger (1939) have repeated the work of Code in testing the recovery of histamine added to blood when alcoholic extraction is used. They have not stated whether they used 10 cc. blood as required

by the Barsoum and Gaddum method or 5 cc. as suggested in the modified procedure. Utilization of the smaller volume of blood, by reducing the quantity of residue, facilitates the extraction. With the use of larger quantities of alcohol and more frequent extraction than originally advised by Barsoum and Gaddum, Anrep et al. have obtained the complete recovery of histamine added to blood. In the present study when prolonged repeated extraction with relatively large volumes of alcohol was used a satisfactory recovery of histamine was also obtained.

SUMMARY

In guinea pigs, rabbits, cats, dogs and one horse and one cow the histamine content of the femoral bone marrow exceeded that of the blood. The histamine in marrow was found associated with the red or cellular portion of the tissue. Pure yellow marrow was free or almost free of histamine. It seems possible that the cells of the blood containing histamine may carry it with them as they leave the marrow. The use of alcohol in the extraction of blood for histamine estimation is discussed.

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ON THE REGULATION OR HOMEOSTASIS OF THE CHOLIC ACID OUTPUT IN BILIARY-DUODENAL FISTULA DOGS[1, 2]

A. L. BERMAN, E. SNAPP, A. C. IVY AND A. J. ATKINSON

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago

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The occurrence of an entero-hepatic circulation of bile salts, first demonstrated by Schiff (1), is established. However, relatively little is known concerning the method by which the cholic acid output is regulated. It has been postulated that some regulatory mechanism must exist in the animal organism which prevents the accumulation of bile salts (2, 3).

In previous studies with bile fistula dogs we have made two observations which appeared of special significance for the regulation or homeostasis of the bile salt output. First, we have noted in 250 experiments on 75 biliary fistula dogs that, if no bile salts are given, the bile salt output on our standard diet attains a remarkably constant level of about 0.46 gram per 8 hour period (3, 4). Second, we observed that, when whole dog bile or bile salts were administered, a fairly constant proportion of approximately 90 per cent could be recovered as "extra" bile salts in the bile (3, 4). If it is true that a constant quantity is synthesized under constant dietary and digestive conditions, and that a constant proportion of the bile salt intake is recovered in the bile during one entero-hepatic circuit, it follows that at some time the amount lost will equal the amount synthesized, and that the bile salt output will be regulated or maintained at a constant level. That is, given an animal fed at 8 hour intervals without the return of bile, if the initial supply of bile salts is low, synthesis will gradually restore a homeostatic level, and if the initial supply is in excess, 10 per cent loss during each entero-hepatic circuit will reduce it to a homeostatic level.

In order to test the above reasoning, we have followed the course of cholic acid output during successive 8 hour periods in biliary-duodenal fistula dogs under the following three conditions: *a*, when the initial dose was only 0.46 gram (the basal output per 8 hr. period, when no bile is returned); *b*, when the initial dose was 2.7 grams, and *c*, when the initial dose was 5.7 grams.

METHODS. Seven biliary-duodenal fistula "suction" dogs were used.

[1] This work has been assisted by the E. L. Dawes Fund.

[2] This work was aided by the E. L. Dawes and the Marjorie Newman Grants.

The animals weighed between 10 and 12 kilos. The biliary fistula was similar to that employed by Rous and McMaster (5), except that the rubber catheter was placed through the cystic duct into the common hepatic duct. A small rubber tube, $\frac{1}{32}$ x $\frac{1}{32}$ inch, was placed into the duodenum, so that it extended caudalward about 6 to 8 inches. Through this tube the bile was returned into the intestine at the rate of 1 cc. per minute. The return bile was allowed to drip into the intestine usually over a period of 3 to 4 hours. To insure the most quantitative results, a small amount of suction was applied continuously to the tubing draining the biliary passages (6, 3). The amount of suction was never greater than 16 inches of water pressure. The animals were kept on suction for 24 hours a day, except for a few minutes at meal time and when they were dressed. They were fed three times a day, and each time they received one-third of the diet of "Pard" and milk (12 per cent protein, 9 per cent carbohydrate, 6 per cent fat, and a supplement of cod liver oil and dried yeast), so that their daily control output of cholic acid would approximate the amount obtained in previous studies.

The *experimental procedure* consisted of the following steps: 1. Several weeks after the operation the animal was standardized to the diet and the return of bile to insure a healthy state. 2. *Basal period.* Then, the animal was fed the diet every 8 hours but no bile was returned for from 3 to 5 days. This gave the basal output of cholic acid on the diet without the return of bile. 3. *The experimental period.* Then, the animal was given the initial dose of cholic acid in the form of dog's bile. At each meal time, or at each 8 hour interval, the bile secreted during the previous period was collected, measured, and 1 to 2 cc. removed for cholic acid analysis. The remaining volume of bile was then returned to the intestine of the animal during the first 3 to 4 hours of the next 8 hour period. Then, at the end of the next 8 hour interval, the animal was fed again, the bile measured, and after removing 1 to 2 cc. for cholic acid analysis the remainder was returned to the dog. This procedure was performed three times a day at 8 hour intervals throughout the length of an experiment. Thus, the bile salts made three entero-hepatic circulations each day.

Kocour and Ivy (6) found that, when food and bile was given every 6 hours, the volume output of bile was very constant. Schmidt et al. (3) noticed no accumulation of cholates when bile and food were fed every 8 hours. Because of these observations we chose to subject the bile salts to three circuits each day.

Calculation of the per cent recovery of cholates. During each period we know the intake and output of cholates. For example, the intake is 3.8 grams and the output is 3.8 grams for an 8 hour period. The output minus the synthesis for a 8 hour period, or $3.8 - 0.46 = 3.34$, gives the output actually obtained from the intake of 3.8 grams; 3.34 divided by 3.8 =

88 per cent recovery of the cholates introduced. Until the introduced cholates are labelled in some way, it must be assumed that synthesis remains approximately constant for each 8 hour period during the absorption of cholates and that the 12 per cent "loss" applies only to the cholates introduced into the intestine. This assumption is implicit in all of our calculations, but does not alter the observed facts or the conclusions.

Results. The averaged data from the three experiments on the different initial doses of cholic acid are shown graphically in figures 1, 2 and 3. It will be seen that in each case the cholic acid output leveled off at

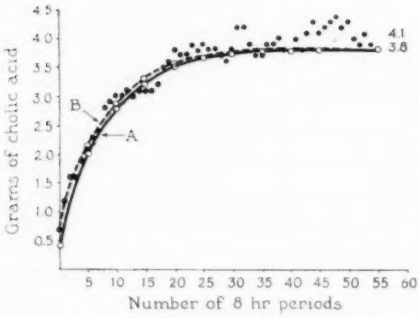

Fig. 1

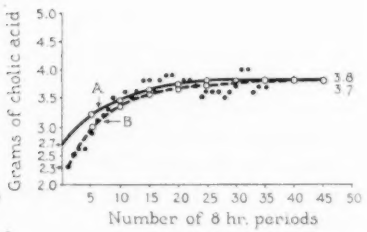

Fig. 2

Fig. 1. Left. This is the average curve of recovery (5 dogs) when the initial return of cholic acid was that amount excreted during the preceding 8 hour period, during which no bile was returned. The dots indicate the experimental recovery of each period. Curve A, the broad-line, represents the curve calculated from $O_n = B^n I + A\left(\frac{B^n - I}{B - I}\right)$, where $B = 0.88$; $I = 0.46$, and $A = 0.46$. Curve B, the broken-line, represents the curve calculated when I is the actual input of the second 8 hour period; that is, the actual initial dose is ignored and the dose given at the second administration is used.

Fig. 2. Right. This is the average curve of recovery (5 dogs) when the initial input of cholic acid was 2.7 grams. Curve A: $B = 0.88$, $I = 2.7$, and $A = 0.46$. See legend of figure 1 for formula and meaning of curve B.

approximately the same point regardless of the initial dose of bile salts. In fifteen tests the cholic acid output leveled off at 4.1 grams per 8 hour period, while in five tests a relatively constant output was reached at 3.7 grams per 8 hour period. When a large dose of cholic acid, such as 5.7 grams, was the initial dose, the curve of the cholic acid output fell to the "*homeostatic level.*" Whereas in the other initial doses, 0.46 gram and 2.7 grams, the curve rose to the "homeostatic level." It can also be seen that, when large doses of bile salts, such as 2.7 and 5.7 grams, were introduced into the intestine at a time when the animal is basal for the diet but no return of bile, the liver or intestine was unable to utilize efficiently the

intake during the first 8 hour period. However, the liver or intestine readjusted itself rapidly, and the cholic acid output either rose or fell gradually to the homeostatic level.

The data have also been analyzed mathematically. In figure 4, the output of cholic acid has been plotted against the input for each individual period of the three experiments. Obviously the points tend to form a straight line, and the data were fitted to the following straight lines, $y = bx + a$ and $x = by + a$. Both regression equations were obtained and the average of the two is plotted on the graph. The correlation

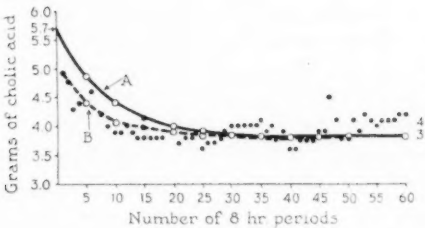

Fig. 3

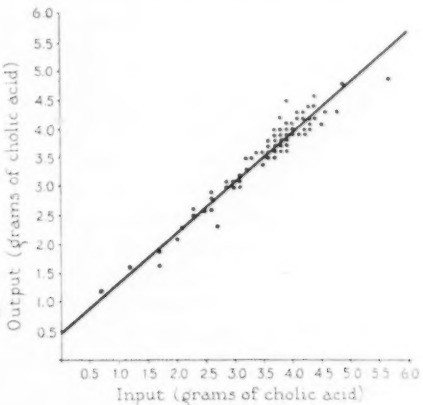

Fig. 4

Fig. 3. Left. This is the average curve of recovery (5 dogs) when the initial return of bile salts was 5.7 grams. Curve A: $B = 0.88$, $I = 5.7$, and $A = 0.46$. See legend of figure 1 for formula and meaning of curve B.

Fig. 4. Right. This shows the relation between output and input of cholates. The dots represent the experimental data. $y = bx + a$; $b = 0.8505$; $a = 0.568$; $y = x = 3.799$. $x = yb + a$; $b = 0.9015$; $a = 0.389$; $y = x = 3.956$. The straight line is the average of the two equations. Average basal output on diet alone per 8 hour period, 0.479 gram; recovery, 87.6 per cent; and homeostatic level, 3.878 grams. Correlation coefficient, 0.94.

coefficient is 0.94, which indicates a high degree of correlation between the input and the output. The final equation which shows the linear relationship between input and output has the following form: Output = input $\times$ b + a where b is the slope = 0.876, and a is the point of intersection on the ordinate = 0.479.

There are several points of significance revealed by this equation. First, the high correlation coefficient (0.94) indicates the relative accuracy of the linear relationship between input and output of cholic acid, within the range studied. It should be mentioned that, if large enough doses are

given diarrhea results and will alter the relationship (3); this level was avoided in the present study. Second, the *a* constant, which is the output intercept, indicates that if no bile salts are administered, 0.479 gram of cholic acid per 8 hour period will be secreted in the bile. From 250 experiments on at least 75 biliary fistula dogs, we have experimentally established the control output on our diet to be 0.46 gram per 8 hour period. Thus, we have a good check of the basal synthesis on our diet. Third, the *b* constant, the slope, reveals that the recovery of administered bile salts is constant in these dogs at about 88 per cent. In order to calculate recovery during the return of bile, one must assume the existence of a synthesis of about 0.48 gram per 8 hours. Calculation of the individual recoveries for each period in the three experiments has given an average recovery of 88 per cent and has revealed no tendency to vary with the dosage. Thus, another good check was obtained. Fourth, the equation reveals that, if small doses are given, the output will exceed the input, and that if large doses are given the output will fall short of the input, but never by more than 12 per cent (unless diarrhea occurs). At some point between these two extremes the input will just equal the output, and solving the equation reveals this level to be 3.8 grams per 8 hours. The average output of cholic acid during the terminal 10 periods in the three experiments, when the animals had apparently leveled out, is 3.9 grams per 8 hours. Fifth, from this it follows that regardless of the initial dose, if the secreted bile is returned, a final level of approximately 3.8 grams should be reached.

The relationship between the initial dose and the course by which 3.8 grams per 8 hours is finally reached can be expressed by the following equation: $O_n = B^n I + A\left(\frac{B^n - 1}{B - 1}\right)$ in which n represents the successive 8 hour periods, O, the output of cholic acid at any period n, B, the percentage recovery of cholic acid administered during any period n, namely, 88 per cent, I, the initial dose, and A, the basal cholic acid output on the diet, or 0.46 gram per 8 hour period. The curves predicted by this equation have been drawn in figures 1, 2 and 3. It will be seen that there is fair agreement between the theoretical curves and the actual data. The broad-line curve, A, is the curve obtained when the level of the initial period of return of bile is used. The broken-line curve, B, is the curve obtained when the second period of return of bile is used. Curve B fits the experimental data best, and is to be preferred because after a 3 to 5 day period of no absorption of bile salts the liver or intestine apparently does not handle the bile salts as it does later; with the higher doses more bile salts are "lost" during the initial period. When the initial dose is low, or is the control output, the two curves are practically identical.

Discussion. The data show definitely that regardless of the initial dose of cholates, the cholic acid output progressively returns to a homeo-

static level which is determined by the basal synthesis of cholic acid from the diet used. When the initial dose of cholic acid introduced into the intestine is less than the dietary homeostatic output per 8 hour period, the cholic acid being circulated accumulates until it reaches the homeostatic level of output. This is because the rate of synthesis exceeds the rate of "loss" per 8 hour period. When the initial dose of cholic acid is greater than the dietary homeostatic output per 8 hour period, the circulating cholic acid diminishes until it reaches the homeostatic level of output. This is because the rate of "loss" per 8 hour period is greater than the synthesis. In either case the "loss" of cholate from period to period is 12 per cent, which will not be exceeded until the "efficiency level" of the liver or intestine appears to be exceeded. This occurs during the first and sometimes the second 8 hour period when bile is returned after a period of privation of bile, and also when large amounts of bile or cholic acid are given which cause catharsis or the "sluice" mechanism of the intestine to operate. When the dietary homeostatic level is reached synthesis apparently balances "loss."

Where the "loss" occurs or what process is involved has not been determined. In fact, when the animal reaches the homeostatic level of output we only know positively that output equals intake. What happens to synthesis and "loss" is unknown. Sythesis and "loss" may be equal, or synthesis may decrease and loss increase. From our observations and those of Smith, Groth and Whipple (7) the quantity and quality of the protein in the diet appear to be the most important factor determining the homeostatic level of cholic acid output. It would appear as though the homeostatic level could be maintained at 2, 3, 4, 5 and possibly 6 grams of cholic acid per 8 hour period, depending on the quantity and quality of the protein fed.

It should be noted that the equation, O_n, employed as it was in the case of curve B in the first three figures, permits the prediction of the cholic acid output for any subsequent period, when the basal output per period of time on the diet is known, the average loss of cholic acid during one entero-hepatic circuit is known, and the animal is healthy and consumes with appetite the diet fed under the conditions of our experiments. The equation also makes certain implications regarding how the homeostatic level of cholic acid output is maintained in the presence of the gall bladder and also following cholecystectomy. These implications, however, must be tested by actual experiments which simulate as closely as possible the cholecystate and acholecystate condition.

SUMMARY AND CONCLUSIONS

Fifteen experiments were performed on seven biliary-suction-duodenal fistula dogs. After adjusting the dogs to an eight-hour feeding schedule without the return of bile to the intestine in order to determine the basal

output of cholic acid on the diet, three different initial doses of cholic acid in the form of cholates in dog bile were introduced into the duodenum, and the output of cholic acid determined for each successive eight-hour period. The bile formed during each preceding eight-hour period, after the initial introduction of bile, was returned to the intestine. This was repeated every eight hours for two or three weeks, the cholic acid output for each eight-hour period being determined from a 2 cc. sample.

When fed the diet we used, the dogs synthesized from 0.46 to 0.48 gram of cholic acid every eight hours, when no bile was returned to the intestine. When a certain quantity of cholic acid in the form of cholates was introduced into the intestine, 88 per cent on the average was recovered in the bile. This recovery was quite constant for various amounts, except when amounts sufficient to cause catharsis were used. When a relatively large amount of bile was introduced into the duodenum after a period of privation of bile, the mechanisms which are exposed to the bile salts were upset during the first and sometimes the second eight-hour period.

The data show that regardless of the initial dose of cholates, the cholic acid output progressively returns under the conditions of our experiment to a homeostatic level of from 3.8 to 4.1 grams per each eight-hour period. This level is chiefly determined by the basal dietary synthesis of cholic acid, since the average percentage "loss" (12 per cent) during each enterohepatic circuit apparently remains constant. When the homeostatic level is reached after about two weeks, synthesis apparently balances "loss" of cholic acid.

An equation is presented which permits the prediction of the cholic acid output for any subsequent period when the basal output per unit of time on the diet is known and the average loss of cholic acid during one enterohepatic circuit is known and the diet and frequency of feeding are kept constant.

We gratefully acknowledge the assistance of Doctors John Gray and F. T. Jung in formulating the equation.

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GLYCOGEN LEVELS IN THE ISOLATED LIVER PERFUSED WITH CORTICO-ADRENAL EXTRACT, INSULIN AND OTHER PREPARATIONS[1]

E. L. COREY AND S. W. BRITTON

With the technical assistance of R. F. KLINE

From the Physiological Laboratory of the University of Virginia Medical School

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Although many years have elapsed since the effects of endocrine extracts were first tested in the laboratory, the influence of such preparations on carbohydrate metabolism is still not at all clear. The confusion found in textbooks and monographs, the great number of original articles and symposial discussions on the subject, all offer testimony to this fact. Some conclusions regarding relationships between the adrenal cortex and carbohydrate metabolism which have been drawn by this school in the past decade have not passed without vigorous contest. With the hope that a simplified, direct attack on the problem might lend clarity to the situation, the effects of various hormones on carbohydrate levels in the isolated liver have now been investigated. Cortico-adrenal extract, desoxycorticosterone and insulin have been tested in particular, together with potassium solutions in the extract series, for their possible influence on the glycogen content of hepatic tissues perfused *in vitro* under rigidly controlled conditions.

METHODS. The animals from which the tissues were derived were lightly anesthetized with ether; the hepatic portal vein was cannulated as rapidly as possible—usually within five minutes—and the entire liver then removed to a constant-temperature bath at 37° containing isotonic Ringer's solution. The liver was perfused through the cannulated vein in different experiments with various solutions which were continuously oxygenated and also maintained at 37°. The portal vein was used as the perfusion route since it appears to supply about 75 per cent of the blood entering the liver; also, it was felt that any possible advantage that might be gained by perfusion through both artery and vein would be vitiated by increased technical complications (in small animals such as the rat and cat) and delay in starting perfusion. The success of the technique was judged mainly by the completeness with which the blood was expelled from the

[1] Grateful acknowledgment is made of aid received from the Committee on Research in Endocrinology of the National Research Council.

liver, as indicated by the quickly fading color of that organ at the outset of perfusion. When any doubt arose regarding the adequacy of the perfusion, the experimental material was discarded.

The perfusate entered the vein at an average pressure of 10 mm. Hg, with occasional brief fluctuations between 8 and 12 mm.; commonly from 10 to 15 cc. per minute were introduced. Bearing in mind that large organs tend to deteriorate rapidly when isolated from the normal circulation, and that under such conditions glycogenolysis is greatly accelerated, the period of perfusion was arbitrarily limited to 15 minutes.

Initial tissue samples were taken as soon as the liver was placed in the bath; the sampling was then repeated at 5-minute intervals. Glycogen concentrations were determined in all cases by a modified Pflüger method (Britton and Silvette, 1932). Animals in various stages of nutrition were purposely included in the study, with the thought that responses to the various perfusates might conceivably vary in livers of high initial glycogenic

TABLE 1

The effect of perfusion of the rat liver for 15 minutes with various solutions

NO. OF RAT LIVERS PERFUSED	PERFUSATE	DECREASE IN GLYCOGEN
		per cent
5	Ringer's solution	75
5	Glucose (5%) in Ringer's solution	35
5	Cortico-adrenal extract (5%) in glucose-Ringer's solution	21

content from those given by organs depleted of carbohydrate material (see tables).

RESULTS. Cat livers were used as experimental material after considerable trial with those from the rat, since glycogenesis could not be definitely demonstrated, within the time limits employed, in the latter animal. Nevertheless, the rat results were of interest. Thus, following perfusion with Ringer's solution, the rat liver showed an average decrease in glycogen content of 75 per cent (table 1) and the addition of glucose to the perfusate reduced this glycogen loss to 35 per cent. Perfusion of rat livers with Ringer-glucose solution to which cortico-adrenal extract was added reduced the loss in glycogen, however, to an average of only 21 per cent. There was thus a decrease of 40 per cent in the glycogenolytic rate when extract was used, in comparison with that of the Ringer-glucose mixture alone.

Although the above experiments gave indirect evidence of glycogenic activity in the presence of cortico-adrenal extract, observations of actual glycogenesis were of course desirable before conclusions could be drawn

TABLE 2

Glycogen concentrations in cat livers following perfusion with various solutions

CAT NO.	LIVER GLYCOGEN (GRAMS PER CENT)				
	Normal	5 minutes	10 minutes	15 minutes	Cc. perfused
			A. Control groups		
			1. Glucose in Ringer's solution*		
1	0.15	0.15	0.14	0.14	700
2	0.28	0.20	0.17	0.11	600
3	0.91	1.09	0.85	0.71	700
4	2.52	2.46	2.45	1.86	900
Average	0.96	0.97	0.90	0.70	725
			2. Glucose in Ringer-gum solution		
5	0.27	0.36	0.29	0.26	175
6	0.45	0.40	0.44	0.46	210
7	0.53	0.57	0.42	0.40	155
8	0.95	1.02	0.77	0.78	220
9	1.14	1.19	0.87	0.65	175
10	1.42	1.99	0.92	0.85	200
11	2.66	2.78	2.36	2.19	200
Average	1.06	1.19	0.87	0.79	191
			B. Experimental groups		
			1. Cortico-adrenal extract in glucose-Ringer-gum solution		
12	0.20	0.32	0.34	0.29	150
13	0.24	0.26	0.70	0.49	155
14	0.31	0.38	0.53	0.66	160
15	0.63	0.77	1.14	0.94	150
16	0.75	0.90	0.79	0.82	190
17	1.55	1.95	2.60	2.59	185
18	1.58	1.84	1.93	2.56	210
Average	0.75	0.92	1.15	1.19	171
			2. Cortico-adrenal extract in potassium acetate-glucose-Ringer-gum solution		
19	0.18	0.15	0.16	0.14	200
20	0.33	0.49	0.36	0.34	220
21	0.79	0.70	0.60	0.52	300
22	0.90	0.74	0.43	0.39	190
23	1.47	1.05	0.60	0.55	210
Average	0.73	0.63	0.43	0.39	224

TABLE 2—*Concluded*

CAT NO.	LIVER GLYCOGEN (GRAMS PER CENT)				
	Normal	5 minutes	10 minutes	15 minutes	Cc. perfused
B. Experimental groups—Continued					
3. Desoxycorticosterone† in glucose-Ringer-gum solution					
24	0.12	0.20	0.16	0.21	175
25	0.72	0.38	0.45	0.39	200
26	1.31	0.88	0.73	0.45	210
27	2.53	2.31	2.46	2.39	180
28	3.19	2.72	2.66	2.43	200
Average	1.57	1.30	1.29	1.17	193
4. Insulin in glucose-Ringer-gum solution					
(a) 1 unit per 100 cc.					
29	2.51	2.44	1.73	1.95	190
30	2.90	2.84	2.40	2.42	200
(b) 2 units per 100 cc.					
31	0.29	0.21	0.22	0.34	210
32	0.33	0.29	0.24	0.22	180
(c) 10 units per 100 cc.					
33	0.73	0.37	0.35	0.39	130
34	0.91	0.75	0.25	0.28	185
35	1.10	0.94	0.93	0.89	155
Average	1.25	1.12	0.87	0.93	179

* Composition of perfusates used:

A-1. Glucose (5 per cent) in Ringer's solution.

A-2. Glucose (5 per cent) in a 7 per cent solution of gum arabic in Ringer's solution.

B-1. Cortico-adrenal extract (5 per cent) in a Ringer solution containing 5 per cent glucose and 7 per cent gum arabic.

B-2. Cortico-adrenal extract (5 per cent) in Ringer's solution in which the potassium chloride was replaced by potassium acetate in 1 per cent concentration.

B-3. Desoxycorticosterone in oil, emulsified in a Ringer solution containing 5 per cent glucose and 7 per cent gum arabic.

B-4. Insulin in the amount indicated in the table, in Ringer's solution containing 5 per cent glucose and 7 per cent gum arabic.

† Desoxycorticosterone acetate ("Cortate") in sesame oil, furnished through the generosity of the Schering Corporation.

regarding specific action of the hormone on the liver. Further experiments were therefore carried out, employing tissues from the cat. The results are summarized in table 2.

It is apparent that no significant glycogenesis was demonstrable in any

of the experiments other than those in which cortico-adrenal extract was utilized in the perfusing fluid. A slight rise in glycogen was observed during the first five minutes of perfusion with the glucose-gum-Ringer solution. This action was transitory, however, the average glycogenic level dropping to 75 per cent of the initial value within 15 minutes (table 1, A2).

Perfusion with a similar solution to which cortico-adrenal extract was added in 5 per cent concentration yielded results indicative of glycogenesis in all instances. Following only five minutes of perfusion an average rise in glycogen content of 22 per cent was observed; the glycogen building rate increased more rapidly thereafter, and at the end of 10 minutes a 53 per cent average increase had taken place. Synthesis of glycogen for the 15 minutes' perfusion period averaged the highest of the whole group (table 2, B1).

Potassium acetate solution given with cortico-adrenal extract produced a strikingly different result: glycogenesis was prevented, and indeed losses of carbohydrate from the liver were observed (table 2, B2). In the average percentage changes in glycogen content from the initial levels shown in figure 1, it is interesting to note that the curves obtained by perfusion with cortico-adrenal extract and extract plus potassium salt are approximately mirror images.

Perfusion of the liver with desoxycorticosterone was surprisingly ineffective in promoting glycogen formation. The average figures for this group showed instead considerable glycogen loss (table 2, B3).

Insulin failed to bring about carbohydrate storage in the liver in all cases; on the contrary, there was an average fall in glycogen content of about 30 per cent during these experiments. Three different concentrations of the hormone were tested (table 2, B4). It is of course possible that longer perfusion periods of different concentrations of insulin might modify these results.

Discussion. The remarkable ability of extracts of the adrenal cortex to build up glycogen in the excised liver, shown in the present paper and an earlier preliminary report (Corey and Britton, 1940), would appear to clinch the arguments of many years past and force the conclusion that the adrenal cortex elaborates a specific glycogenetic hormone. To this belief most of the results of this laboratory have indeed led for more than a decade. It is now observed that this glycogen-forming or glycopexic hormone of the adrenal cortex is able to function as an independent, specific agent, on the isolated organ. It is able to act directly and rapidly and may raise the carbohydrate content of the liver 50 or even 100 per cent in 10 or 15 minutes. Further, it is effective in the presence of low or even high initial liver glycogen concentrations; in one case the value rose from 0.24 to 0.70 gram, and in another from 1.55 to 2.60 grams per cent,

each within a 10-minute experimental period. A report by Seckel (1940) indicates that glycogenolysis in the excised liver may be inhibited by extracts of the adrenal cortex.

Addition of potassium acetate to the perfusing fluid apparently rendered cortico-adrenal extract, present in the same solution, inactive as far as glycogenetic activity is concerned. When the percentage changes in glycogen concentration are considered (fig. 1), it is apparent indeed that potassium perfusion resulted in the lowest hepatic glycogen levels which

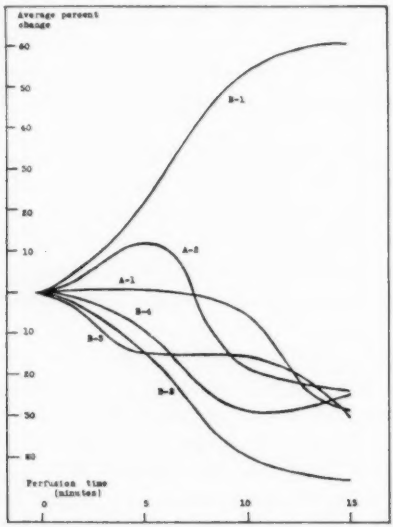

Fig. 1. Showing the average percentage change in the glycogen content of perfused cat livers. The legends correspond to the various divisions of table 1, thus: *A-1*, glucose-Ringer's solution; *A-2*, glucose-gum-Ringer; *B-1*, cortico-adrenal extract in glucose-Ringer-gum; *B-2*, cortico-adrenal extract in potassium-acetate-glucose-Ringer-gum; *B-3*, desoxycorticosterone in glucose-Ringer-gum; *B-4*, insulin in glucose-Ringer-gum solution.

were encountered. A definite antagonism is thus indicated between the action of the potassium salt and the cortico-adrenal hormone. Membrane or osmotic effects may of course be involved, as discussed in an earlier paper (Britton, Silvette and Kline, 1938).

Inability to demonstrate hepatic glycogenesis in any of the control or other experiments is in contrast very striking. Tests with different concentrations of insulin (1, 2 and 10 units per 100 cc. perfusing fluid) which were carried out appear to be definitely negative. Under similar conditions cortico-adrenal extract was highly effective.

Desoxycorticosterone also produced no positive influence on the liver glycogen levels *in vitro*, which is in keeping with correlated observations in this and other laboratories. This substance obviously lacks, therefore, an important (glycogenetic) constituent found in whole extracts of the adrenal cortex. It is of special note, on the other hand, that when the isolated liver is perfused with whole cortico-adrenal extract it is able to build up promptly large amounts of glycogen. This insulin fails to do. Considered in relation to somewhat similar results on the intact animal given in the following paper (Britton and Corey, 1941), these findings appear to be highly significant.

SUMMARY

Cortico-adrenal extract perfused through the rat liver with glucose proved more effective in the prevention of glycogenolysis than did glucose alone.

In the perfused cat liver *in vitro* large increases in glycogen content were brought about by the use of cortico-adrenal extract with Ringer-gum-glucose solution. Elevations of 50 to 100 per cent above the pre-perfusion glycogen levels occurred in some instances within 10 or 15 minutes. No other perfusates tested had this effect; in contrast, there usually occurred marked diminution in the glycogen values.

Addition of potassium acetate to perfusion fluids containing cortico-adrenal extract prevented the glycogenetic action of the extract.

Desoxycorticosterone acetate did not stimulate glycogenesis in the isolated cat liver.

Glycogenesis could not be demonstrated in cat livers perfused with glucose solutions containing insulin in the several different concentrations employed in these experiments. Instead there occurred an average glycogen decrease of about 30 per cent under insulin dosage, while an average rise of 60 per cent was effected by extracts of the adrenal cortex.

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PANCREATIC AND CORTICO-ADRENAL INVOLVEMENT IN CARBOHYDRATE REGULATION[1]

S. W. BRITTON AND E. L. COREY

With the technical assistance of R. F. KLINE and C. R. FRENCH

From the Physiological Laboratory of the University of Virginia Medical School

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Toward the end of an extended review written ten years ago it was stated that the adrenal cortex was apparently "concerned with the storage and utilization of carbohydrates, and possibly with some phase of protein metabolism" (Britton, 1930). Shortly after this it was shown that extracts of the adrenal cortex produced hyperglycemia in normal and adrenalectomized animals, and that the blood-sugar-raising ability was a direct function of the amount of hormone injected and of the elapsed time (Britton and Silvette, 1931a). The effects of cortical preparations were produced by oral as well as other modes of administration (Britton and Silvette, 1931b). Evidence of remarkable increases in liver and muscle glycogen, effected by cortico-adrenal extract in normal and adrenalectomized animals, was also brought forward. The terminal stages of adrenal insufficiency were characterized in most animal types by severe hypoglycemic convulsions, it was observed, and restoration from extreme prostration by adrenal extract was found to synchronize with increases in blood glucose (Britton and Silvette, 1932). In face of much opposition to this earlier work the Virginia school steadily extended and corroborated these results, and within the past few years rather general support for their observations has been advanced.

METHODS. The relative activities of the pancreas and adrenal cortex in carbohydrate regulation have been particularly investigated in the present studies. Carbohydrate levels in adrenalectomized, pancreatectomized, and adreno-pancreatectomized cats have been determined under different experimental conditions. Adrenalectomy and pancreatectomy were performed in single-stage operations; adreno-pancreatectomy was carried out in two stages in earlier work, but equally good results were achieved in later experiments with a single quick operation, lasting about 15 minutes.

The influence of glucose feeding and injection, in some cases accom-

[1] Grateful acknowledgment is made of aid received from the Committee on Research in Endocrinology of the National Research Council.

panied by insulin or cortico-adrenal extract, has been noted and compared with conditions in operated and normal untreated animals. Some early tests were made of the effects of the hormone preparations alone, but it became apparent that the utilization of glucose served to emphasize greatly the glycogenetic possibilities. In different series the effects of the hormones over periods of a few days as well as over several hours have been observed. Glucose solutions were made up in normal saline.

Analyses of blood glucose were made by the Folin-Malmros method (1929), and glycogen by a modified Pflüger procedure (Silvette and Britton, 1932). Three samples were taken from the heart for analysis—one

TABLE 1

Carbohydrate levels in adrenalectomized cats given glucose orally over various periods
(Glucose, 5 per cent solution in 0.9 per cent saline, 7 per cent body weight, twice daily)

CAT NO.	PERIOD OF TREATMENT	BLOOD SUGAR	LIVER GLYCOGEN	MUSCLE GLYCOGEN	HEART GLYCOGEN		
					Apex	Body	Base
	days	*mgm. per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	2	84	0.26	0.15	0.20	0.15	0.22
2	2	59	0.12	0.44	0.36	0.37	0.34
3	2	80	0.43	0.25	0.65	0.45	0.67
4	3	115	0.22	0.21	0.19	0.15	0.14
5	3	60	0.11	0.13	0.29	0.26	0.21
6	5	64	0.15	0.25	0.33	0.35	0.32
7	5	91	0.29	0.48	0.39	0.36	0.32
8	5	62	0.51	0.47	0.37	0.54	0.34
9	6	98	0.35	0.61	0.38	0.48	0.38
10	6	77	0.17	0.35	0.44	0.46	0.50
Controls,* 10 cases, 2–6 days, averages		88	3.31	0.53	0.86	0.86	0.76

* Unoperated animals treated similarly with glucose.

each from the apex, body and base of the organ—but the small differences which were noted in the values led later to one sample only being used, from the thick mid-wall of the left ventricle.

RESULTS. *Adrenalectomized cats.* The effects on carbohydrate levels of oral administration of glucose in saline solution over different periods of days are shown in table 1. Unoperated control cats thus treated with glucose stored up large quantities of glycogen in the liver, and skeletal and cardiac muscle. Adrenalectomized animals treated similarly showed very low carbohydrate levels in all tissues; even after the long-continued (6-day) periods of glucose administration, no significant glycogen storage occurred. Similar negative results have been noted repeatedly after intraperitoneal

injection of glucose solution over shorter periods. During the experiments, it should be observed, the operated animals remained in apparently good condition.

Cardiac glycogen levels for different parts of the heart are given. All indicate the severe disability of the adrenalless animal to synthesize

TABLE 2

Effects of insulin given with glucose on carbohydrate levels under different conditions

CONDITIONS	CAT NO.	BLOOD SUGAR	LIVER GLYCOGEN	MUSCLE GLYCOGEN	HEART GLYCOGEN
		mgm. per cent	*per cent*	*per cent*	*per cent*
A. *Adrenalectomized cats*					
200 mgm. glucose + 2 units per kilo insulin per hour	11	62	0.13	0.37	0.32
	12	50	0.41	0.50	0.60
	13	51	0.12	0.43	0.65
300 mgm. glucose + 2 units per kilo insulin per hour	14	54	0.18	0.61	0.79
	15	51	0.39	0.36	0.57
300 mgm. glucose + 1 unit per kilo insulin per hour	16	54	0.18	0.42	0.38
	17	67	0.21	0.29	0.54
	18	71	0.42	0.41	0.44
B. *Adreno-pancreatectomized cats*					
300 mgm. glucose + 1 unit per kilo insulin per hour	21	71	0.14	0.26	0.14
	22	56	0.27	0.32	0.17
	23	69	0.20	0.34	0.36
500 mgm. glucose + 1 unit per kilo insulin per hour	24	119	0.33	0.29	0.19
	25	121	0.15	0.36	0.58
	26	76	0.38	0.13	0.15
	27	106	0.43	0.29	0.13
600 mgm. glucose + 1 unit per kilo insulin per hour	28	90	0.20	0.45	0.25
	29	88	0.53	0.60	0.28
	30	76	0.20	0.60	0.32
	31	88	0.27	0.47	0.36

Animals were tested for periods of three, four or five hours. Heart glycogen figures quoted represent averages for apex, body and base samples of the organ.

glycogen in this highly important organ, even in the presence of more than adequate glucose supplies.

Insulin was tested on adrenalectomized animals in different concentrations and with different amounts of glucose. Frequently, in preliminary tests, severe convulsions appeared within an hour or so after injection, and smaller doses of insulin with relatively large amounts of glucose were then employed. It will be observed (tables 2 A, 3) that, in the absence

of the adrenals, no hepatic glycogenesis occurred under insulin action. This was true in both short-term experiments extending over a few hours, and long-term tests over several days. Small increases in skeletal muscle and cardiac glycogen are nevertheless indicated.

The ability of the cortico-adrenal hormone to bring about synthesis of glycogen in adrenalectomized animals was readily apparent in all experiments. It is necessary to refer only to the average figures obtained

TABLE 3

Carbohydrate values under different experimental conditions (cats)

CONDITION	TREATMENT	NO. OF ANIMALS USED	BLOOD SUGAR	LIVER GLYCOGEN	MUSCLE GLYCOGEN	HEART GLYCOGEN
			mgm. per cent	*per cent*	*per cent*	*per cent*
Normal	None	10	88	1.22	0.43	0.61
	Glucose-treated*	10	88	3.31	0.50	0.83
Adrenalectomized	Untreated	10	57	0.07	0.21	0.21
	Glucose-treated*	10	80	0.26	0.33	0.35
	C-A extract + glucose	6	151	1.77	0.54	0.66
	Insulin + glucose†	8	57	0.25	0.42	0.54
	Insulin + glucose‡	6	80	0.37	0.37	0.36
Pancreatectomized	Untreated	5	392	0.72	0.44	0.80
	Glucose-treated	5	317	0.99	0.36	0.85
	C-A extract + glucose	5	292	1.01	0.37	0.63
	Insulin + glucose	5	98	0.88	0.47	0.74
Adreno-pancreatectomized	Untreated	10	73	0.14	0.46	0.43
	Glucose-treated	6	224	0.25	0.38	0.45
	C-A extract + glucose	5	193	0.81	0.40	0.71
	Insulin + glucose	11	87	0.28	0.37	0.27

* Glucose solution orally (see details, table 1) for periods up to 6 days.

† Tests made for periods up to 5 hours (table 2).

‡ Tests for 2 and 3-day periods.

Glucose was given in 3 per cent solution, 1 per cent body weight per hour for 4 to 6 hours; 5 to 10 cc. of cortico-adrenal extract were given per hour. Note exceptions above and further details in text.

in this group (table 3). In some cases liver glycogen values up to 2 or even 3 per cent were found after cortico-adrenal treatment.

Pancreatectomy. Untreated pancreatectomized cats with symptoms of weakness showed, along with the usual hyperglycemia, very little change from the normal values for liver, muscle and heart glycogen. Glucose and glucose-insulin treated animals showed slightly augmented liver glycogen levels. Administration of cortico-adrenal extract with glucose

also resulted in increased liver glycogen concentration, and maintenance of the hyperglycemic condition (table 3). The relatively small changes in glycogen in the liver and muscle tissues of pancreatectomized animals under different conditions may be emphasized.

Adreno-pancreatectomized animals. When the adrenals and pancreas were removed in cats, it was particularly notable that the liver glycogen fell to a very low level, and the blood-glucose concentration also was sometimes below normal. In many respects the animals were similar to those which had been adrenalectomized; they developed a comatose state, much like that of adrenal insufficiency, and survived only a few days if untreated. An animal could be restored time after time from the prostrate condition, however, by cortico-adrenal extract; the blood sugar was raised by such injections, and life could be maintained for at least a week or two, and probably much longer if desired. Glucose solutions also restored adreno-pancreatectomized cats showing symptoms of insufficiency, but only temporarily, for periods of a few hours. The following protocols are illustrative:

Protocol: Adreno-pancreatectomy.
Cat 35. Weight 1.60 kilos, female.
April 11, 1939, 10:40 a.m., pancreas and both adrenals removed.
12, 10 a.m., blood sugar 84 mgm. per cent; animal weak; gave 15 cc. cortico-adrenal extract.
13, 9 a.m., blood sugar 200 mgm.; condition good; 10:00 a.m., 10 cc. extract; 11 p.m., 10 cc. extract.
14, 9 a.m., good condition; 10 a.m., blood sugar 238 mgm., 10 cc. extract; 12 noon, blood sugar 256 mgm.
15, normal.
16, normal; blood sugar 250 mgm.
17, 9:45 a.m., blood sugar 208 mgm.; good condition; gave 10 cc. extract; 11:45 a.m., blood sugar 247 mgm.; 1:45 p.m., blood sugar 250 mgm.; tested insulin effect, gave 1 unit per kilo; 4:15 p.m., 2 u.p.k. insulin; 6:10 p.m., blood sugar 95 mgm.; weak; 7:20 p.m., blood sugar 61 mgm.; cat dying; took final samples. Liver glycogen, 0.69 per cent; muscle glycogen, 0.48 per cent; heart glycogen, 0.81 per cent.

Protocol: Adreno-pancreatectomy.
Cat 36. Weight 1.80 kilos, male.
April 11, 1939, 10 a.m., adrenals and pancreas removed.
12, 9:50 a.m., blood sugar 57 mgm. per cent; cat weak; given 36 cc. 3 per cent glucose solution intraperitoneally (2 per cent body weight); 10:50 a.m., improved; repeated glucose injection; 12:05 p.m., blood sugar 255 mgm.; 12:45 p.m., weak again; gave 15 cc. cortico-adrenal extract in 0.9 per cent saline, i.p.; 1:45 p.m., blood sugar 244 mgm.; slight improvement; 10 cc. extract; 5 p.m., much improved; 10 cc. extract; 10 p.m., condition normal.
13, 9 a.m., blood sugar 333 mgm., normal condition; 9 p.m., 10 cc. extract.
14, 10 a.m., blood sugar 263 mgm.; normal; 10 cc. extract; 10 p.m., condition good, no extract.

15, 8:30 a.m., blood sugar 250 mgm.; cat normal; no treatment; 9:45 p.m., same condition.

16, 7:45 a.m., blood sugar 200 mgm.; condition good; 6 p.m., blood sugar 190 mgm., slightly weak, gave 10 cc. extract; 7 p.m., blood sugar 222 mgm.; cat much stronger.

17, 9:45 a.m., blood sugar 220 mgm.; good condition; 10 cc. extract; 4:45 p.m., blood sugar 278 mgm.; 1:40 p.m., blood sugar 266 mgm.; tested insulin action, 1 unit per kilo; 4:15 p.m., blood sugar 250 mgm.; condition normal; gave insulin 2 u.p.k.; 6:10 p.m., blood sugar 86 mgm., cat weak; 7:20 p.m., blood sugar 54 mgm.; animal apparently dying. Took final tissue samples: liver glycogen, 1.23 per cent; muscle glycogen, 0.61 per cent; heart, 0.64 per cent.

While the blood sugar was raised in adreno-pancreatectomized animals by glucose injection, no significant changes in glycogen levels were observed. Furthermore, insulin given along with glucose was unable to influence the liver, muscle and cardiac glycogen values. Various amounts of glucose with insulin were used without positive effect (table 2, B). Adreno-pancreatectomized cats were markedly hypersensitive to insulin injections, it may be noted, and after many tests small doses only were utilized. The pancreatic hormone did not restore or alleviate the condition of insufficiency observed in these animals (see protocols).

Cortico-adrenal extract given with glucose affected notably the liver and cardiac glycogen in adreno-pancreatectomized cats, raising both to approximately normal concentrations (table 3).

Discussion. The present paper together with the preceding (Corey and Britton, 1940) confirm and amplify earlier work from this laboratory emphasizing the rôle of the adrenal cortex in carbohydrate regulation. For several years after the results of the senior author and his colleagues had been presented (1930 onwards), there ensued vigorous discussion regarding the validity of their observations. Swingle (1933), Parkins *et al.* (1936), Grollman and others offered opposing evidence. In an extended monograph and again in a later paper (1936, 1938), Grollman affirmed that the cortico-adrenal hormone did not affect carbohydrate metabolism. Attention at this time turned more particularly to the electrolyte disturbances in adrenal insufficiency.

Recently, with the greater availability of potent extracts of the adrenal cortex as well as synthetic preparations, emphasis has again been given to the possible influence of the adrenal cortex on carbohydrate metabolism. Many investigators have now confirmed our earlier results, and attached greater importance to the carbohydrate complications in experimentally produced adrenal insufficiency as well as in Addison's disease. The numerous experiments of Long and his colleagues (1940), and Verzàr (1939) are of particular note. Anderson et al. (1939) observed that the blood sugar and liver and muscle glycogen were all reduced as early as 24

hours after adrenalectomy in the rat. Uyldert and co-workers (1939) found further in the dog that before the clinical symptoms of adrenal insufficiency appear, there occurs a decrease in the amount of sugar in the blood.

The striking ability of cortico-adrenal extract to raise tissue glycogen levels in adrenalectomized, pancreatectomized and adreno-pancreatectomized animals is demonstrated in the present paper. This sets the adrenal cortex apart in a distinct class as a glycogenetic factor. Insulin in contrast showed relatively little effect, and even in pancreatectomized animals it did not raise the liver glycogen to levels equal to those produced by cortico-adrenal extract. There was evidence in a few cases, however, that insulin brought about some increase in muscle glycogen. A consideration of

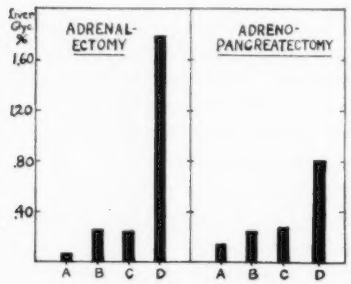

Fig. 1

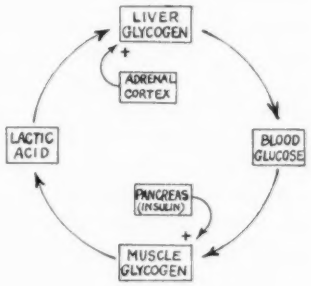

Fig. 2

Fig. 1. Left. Hepatic glycogen levels in adrenalectomized and adreno-pancreatectomized cats under different conditions. A, untreated; B, glucose-treated; C, insulin plus glucose; D, cortico-adrenal extract plus glucose. The marked action of the adrenal hormone in elevating liver glycogen is apparent.

Fig. 2. Right. Diagram indicating the preponderating effects of the cortico-adrenal and pancreatic hormones on the carbohydrate cycle.

glycogen levels in adrenalectomized and adreno-pancreatectomized cats in different experimental tests, shown in the accompanying graph (fig. 1), demonstrates the preponderating influence of the cortical hormone on hepatic glycogen levels.

The restoration by cortico-adrenal extract of adrenalectomized or adreno-pancreatectomized animals showing symptoms of insufficiency would appear to be dependent, at least in part, on an increase in circulating blood glucose. The injection of glucose solution alone was also temporarily effective in both operated groups, while insulin was of no avail.

That the adreno-pancreatectomized animal deteriorates primarily because of lack of cortico-adrenal tissue appears evident. Most of the symptoms which are observed a few days after operation are similar to

those found in adrenal insufficiency, and the carbohydrate losses strongly indicate the dominant effect of cortical removal.

There is still a great deal of confusion regarding insulin action in the body, although it appears an accepted fact that augmented oxidation of carbohydrates occurs under its influence. In Macleod's text (1938) it is observed that in normal (as well as diabetic) animals, "carbohydrate storage" is promoted by insulin injection; Wiggers (1939) notes that this hormone increases glycogen deposition in muscle; and Best and Taylor (1939) remark that liver glycogen is increased by insulin in normal rabbits, probably as a secondary effect due to adrenalin liberation.

In a recent review, Cori (1940) states that insulin is able to effect glycogen formation in liver and muscle, but is not indispensable for the reaction glucose → glycogen. No mention is made of any influence of the adrenal cortex, although one section is devoted to the "effect of hormones on carbohydrate metabolism." Soskin (1940) remarks in another review on carbohydrate metabolism that "it may be supposed . . . that the endocrine (carbohydrate) balance consists of the opposing influence of the hormones of the pancreas and of the anterior hypophysis."

Although insulin has in the past been given the chief place in discussions of carbohydrate metabolism, it is rather amazing that little or no attention has been given to the adrenal cortex in such articles as the above. In contrast Kendall (1940), in discussing the adrenal cortex, placed at the head of its list of functions the regulation of carbohydrate metabolism. A key position for the adrenal cortex in the carbohydrate cycle is indicated (fig. 2) by the results given herein and those we have advanced for many years past.

SUMMARY

The influence of the adrenal cortex is highly important in regulating carbohydrate levels in the body. Comparison of blood sugar and liver, skeletal muscle and heart glycogen concentrations in adrenalectomized, pancreatectomized and adreno-pancreatectomized cats under different conditions points directly to this conclusion. The effects of cortico-adrenal extract and insulin in conjunction with glucose administration have been tested in all series.

Adrenalectomized cats show very severe losses in blood glucose and liver, muscle and cardiac glycogen when untreated. They are able to form only slight amounts of glycogen from glucose given by mouth over periods up to 6 days. No increase in liver glycogen occurs when insulin is given with glucose, but small rises in muscle and cardiac glycogen may occur. Large increases in blood glucose and all glycogen values are produced by extracts of the cortex used with glucose.

Pancreatectomized animals when untreated show practically normal

amounts of liver, skeletal and cardiac muscle glycogen almost up to the time of death. They are able to form liver glycogen from glucose solution alone. Comparatively, somewhat more muscle glycogen appears to be deposited under the influence of insulin, and slightly more liver glycogen under cortico-adrenal extract.

Adreno-pancreatectomized cats which are untreated often show low blood sugar and liver glycogen levels when symptoms appear, somewhat similar to the conditions observed in adrenal insufficiency. They may be restored from severe symptoms time after time, and life may be greatly prolonged, by the injection of cortico-adrenal extract. Such resuscitation is accompanied by an increase in blood sugar. Glucose also brings about temporary restoration. Administration of glucose solution alone or with insulin does not affect significantly the formation of glycogen in adreno-pancreatectomized animals. Cortico-adrenal extract given with glucose brings about large deposits of glycogen in the liver and cardiac tissues.

Cortico-adrenal extract is observed to be well able to form liver glycogen in the absence of the pancreas; insulin does not do so in animals following loss of the adrenal cortex. Apparently the carbohydrate hormone of the cortex stimulates markedly hepatic glycogenesis, while insulin may affect favorably the formation of glycogen in muscle.

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OBSERVATIONS CONCERNING THE PRESSOR SUBSTANCE PRESENT IN THE ISCHEMIC KIDNEY BLOOD OF THE DOG

MEYER FRIEDMAN, ARTHUR SELZER AND JOHN J. SAMPSON

From the Cardio-Vascular Research Department, Mount Zion Hospital, San Francisco, California

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Although many advances have been made in the study of arterial hypertension of renal origin since the pioneering studies of Goldblatt and his associates (1), the exact nature of the mechanism that underlies the elevation of blood pressure following the production of renal ischemia is still unknown. It seems that renal ischemia produces an elevation of blood pressure which is independent of primary changes in the nervous system (2, 3, 4, 5), the heart (6), the circulating blood volume (7), and the excretory efficiency of the kidney (8).

The work of Houssay and his associates (9) demonstrated clearly that the ischemic kidney produced a humoral agent capable of raising the blood pressure of the dog. The presence of renin in the venous blood of both the isolated kidney perfused under reduced pulse pressure and the intact, partially ischemic kidney was reported by Page and his associates (10, 11). A pressor substance was reported by Taquini (12) and by Prinzmetal and his associates (13) to be present in the completely ischemic kidney. It has just come to our attention that Braun-Menendez and his associates (14) have reported the presence of a pressor substance in the venous blood of partially ischemic dogs' kidneys which they believed was identical to the substance described as "hypertensin" by Taquini. However, this last substance is not renin but a product resulting from the reaction between renin and a pseudoglobulin of normal blood. Its chemical, pharmacological and physiological properties appear identical to those of the substance "angiotonin" described by Page and Helmer (15).

In the present communication, the presence of a pressor substance in the venous blood of both the isolated perfused kidney and the partially ischemic but intact kidney of the hypertensive dog is reported. Further, some of the properties of the pressor substance found in this ischemic kidney blood are described. Although the pressor substance found to be present in the ischemic kidney blood of our experiments has many properties similar to those ascribed to "hypertensin" and its apparent counterpart, "angiotonin", it nevertheless does not appear to us to be identical to either.

Throughout the remainder of this report, frequent references will be made to the renal pressor substance, but it must be emphasized that this pressor quality of ischemic kidney blood may be due to a mixture of several substances. Also, in this report the terms renal pressor substance and ischemic kidney blood will be used interchangeably because the pressor constituent of our samples of ischemic kidney blood has not yet been isolated from the blood plasma.

I. THE PRESSOR SUBSTANCE PRESENT IN RENAL BLOOD PERFUSATE. *Methods:* The isolated, freshly removed kidney of a normal dog was perfused with 500 to 700 cc. of normal blood (heparinized) obtained from the femoral artery of another normal dog. A constant nitrogen escape

TABLE 1

Pressor effect of perfused blood

EXPERIMENT NUMBER	PERFUSION OF KIDNEY AND SPLEEN WITH BLOOD						EFFECT OF PERFUSED KIDNEY AND SPLEEN BLOOD ON B.P. OF NEPHRECTOMIZED DOG			
	Organ perfused	Total blood perfused	Renal flow	Duration of perfusion	Number of circulations	Perfusion pressure	Total perfused blood given	Effect of perfused blood on B.P.	Time interval before B.P. change	Duration of B.P. change after injection of perfused blood
		cc.	*cc./min.*	*min.*		*mm. Hg*	*cc.*	*mm. Hg*	*min.*	*min.*
1-A	Kidney	600	9.0	233	2	145	150	+60	<1	19
2-B	Kidney	600	5.9	167	2	170	120	+85	<1	23
3-C	Kidney	350	3.5	83	1	145	75	+45	<1	20
4-D	Kidney	500	6.0	95	2	120	80	+30	<1	10
5-E	Kidney	500	3.0	25	1	150	80	+30	<1	24
Average.....		510	5.48	120.6	1.6	146	101	+50	<1	19.2
6-F	Spleen	500	7.75	142	3	120	100	+8	4-5	<1
7-G	Spleen	500	2.5	53	1	130	50	−10	2-3	<1

apparatus was used which effected a constant, non-pulsatile pressure upon the reservoir in which the blood to be perfused was contained.

The isolated kidney to be used for the perfusion was always immersed in a saline water bath maintained at 37°C., immediately after its removal from a living dog. The renal artery was connected by a cannula to the pressure reservoir containing the arterial blood, and the renal vein was cannulated for the collection of the venous blood. A wide and shallow pan was used for the collection of the venous blood. This pan was constantly but gently agitated to ensure a maximum aeration of the blood before it was again returned to the pressure system for recirculation through the kidney. The entire perfusion apparatus was so designed that the

recirculation of any sample of perfusate could be done without any interruption of the maintenance of a constant pressure. The rate of renal flow, the duration of the perfusion, and the perfusion pressures maintained are given in table 1. Five perfusions were performed.

After the completion of the renal perfusion, samples of perfusate (75–150 cc.) were given at the rate of 10 cc. per minute by vein to nephrectomized dogs (10–15 kgm.) maintained under anesthesia with pentobarbital sodium. In the recipient dog, the mean blood pressure was determined by cannulation of the right carotid artery. Before the introduction of the renal blood perfusate, a control infusion of normal blood equal in

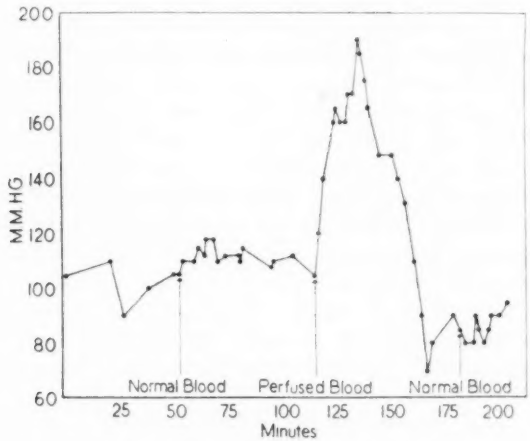

Fig. 1 (Expt. 2-B). The effect of perfused renal blood on the blood pressure of a nephrectomized animal is shown. The effect of the same blood before renal perfusion is also shown. It was given both before and after the introduction of perfused renal blood. One hundred twenty cubic centimeters introduced within 12 minutes were given in each administration.

quantity to the perfusate to be injected was first given. Two blood perfusions of isolated spleens were also performed.

Results: The employment of a constant perfusion pressure caused a marked reduction in renal blood flow in the isolated kidney (see table 1), despite the fact that the average perfusion pressure in the five experiments was 145 mm. Hg.

The blood perfused through the isolated kidney in the manner described above exhibited a strongly pressor effect when given to a nephrectomized dog in contrast to the negligible effect observed when equal quantities of normal blood were given. Thus (see table 1 and fig. 1), in five experiments, the average administration of 101 cc. of renal blood perfusate provoked a

rise of 50 mm. of Hg in the blood pressure of the recipient dog. The pressor effect began in less than one minute and lasted for over 19 minutes in the five experiments. In one experiment (5-E), the perfusion was maintained for only 25 minutes and the blood was not recirculated. Nevertheless, the blood sample contained the pressor substance noted in the other four perfusates. Finally, it may be observed that blood perfused through the spleen in the same manner as described above produced no significant pressure rise when given to a recipient dog.

II. THE PRESSOR SUBSTANCE PRESENT IN THE ISCHEMIC KIDNEY BLOOD OF THE DOG. Because it was observed that blood obtained from a kidney perfused with normal arterial blood for as little as 25 minutes possessed a pressor effect, it was thought advisable to observe the effects of renal blood leaving intact kidneys with a considerable reduction in renal artery flow upon the blood pressure of the same dog and also upon another dog.

Methods: In these experiments, dogs were anesthetized with pentobarbital sodium and before any other procedure was executed, blood pressure readings were taken in the manner described above until a stable control level had been reached. Then each kidney was isolated, a Goldblatt clamp was attached, tightened to complete obstruction and then released sufficiently to permit a small blood flow to the kidney which was always determined by direct inspection. After the adjustment of the clamps, the incisions were rapidly closed and the pressure of the dogs was recorded continuously for the next 4 to 5 hours. In three dogs one kidney was removed and the kidney remaining was clamped as described above.

After periods of time varying from 172 to 338 minutes, the dogs were given 4 to 5 cc. of purified heparin (Connaught) and reoperated. The renal vein of one ischemic kidney was then cannulated and the flow was collected and measured. The rate of flow varied from 10 to 20 cc. per minute and the average time taken for the collection of the renal venous blood was 45 minutes, and about 500 to 600 cc. of blood were obtained in a single experiment. Samples of this blood were then given to previously nephrectomized dogs. Control infusions of normal blood were given to the recipient dogs both before and after the infusion of the blood from the partially ischemic kidney. Nine experiments were performed.

Results: The pressor effect of acute partial renal ischemia on the same dog. Of the nine dogs whose kidneys or kidney (the other being removed) were made partially ischemic, seven exhibited a fairly immediate rise in their blood pressures varying from 15 to 40 mm. Hg above the control pressure level (see table 2 and fig. 2). In the two dogs (3-L, 8-A) not showing a rise, considerable hemorrhage was observed to have occurred.

The pressor effect of blood from a partially ischemic kidney on the nephrectomized dog. As can be seen in table 2, the ischemic kidney blood when

given intravenously to nephrectomized dogs (10–15 kgm.) effected a rapid rise of blood pressure in each of the nine experiments, the average rise being 26 mm. of Hg. Further, the ischemic kidney blood was similar to the renal blood perfusate in the rapidity and duration of its action. The greater rise following the introduction of the renal blood perfusate may well be due to the larger amounts of blood given and not due to any essential difference in the pressor substance contained in the blood samples. The introduction of normal blood into the nephrectomized dogs in quan-

TABLE 2

Pressor effect of blood from partially ischemic kidney

DOG NUMBER	PARTIAL ISCHEMIA OF KIDNEY IN DOGS AND COLLECTION OF ISCHEMIC KIDNEY BLOOD							EFFECT OF ISCHEMIC RENAL BLOOD ON B.P. OF NEPHRECTOMIZED DOG			
	Release of left clamp	Release of right clamp	Maximum B.P. rise in a dog with ischemic kidneys	Time for beginning of rise	Duration of partial ischemia before renal flow collection	Rate of renal flow	Duration of renal flow collection	Total of ischemic renal blood given	Effect of ischemic renal blood on B.P.	Time interval before B.P. change	Duration of B.P. change after injection of ischemic renal blood
	turns	*turns*	*mm. Hg*	*min.*	*min.*	*cc./min.*	*min.*	*cc.*	*mm. Hg*	*min.*	*min.*
1-J*			+40	9	220	11	69	70	+20	<1	27
2-P			+40	106	248	19.7					
3-L†			0		198						
4-M	- ½	¾	+15	190	338	9.8	52	100	+30	<1	11
5-S	¼	0	+22	85	172	15	34	55	+30	<1	17
6-V	⅛	¼	+20		240	6.1	40	100	+12	<1	1§
7-W*	¼		+23	22	224	12.2	30	50	+27	<1	23
8-A‡	⅜	¼	0		213	12.0	60	60	+23	<1	21
9-B	⅜	⅜	+25	250	266	11.8	75	50	+40	<1	10
Average			20.5	110.3	235.4	12.2	45	60.6	+26	<1	15.7

* One kidney removed.

† Dog had hemorrhage.

‡ Dog went into profound shock.

§ Recipient dog was not bilaterally nephrectomized.

tities and at infusion rates comparable to those used in the introduction of partially ischemic kidney blood did not provoke a significant blood pressure change in the recipient.

The pressor properties of ischemic kidney blood were compared with those of renin in a series of 13 nephrectomized dogs. In the seven dogs receiving an average injection of 2.5 mgm. of purified renin there was an average rise in their blood pressures of 40 mm. Hg. The maximum elevation of pressure occurred six minutes after the injection of renin and the pressor effect remained for over 29 minutes. The average introduction of

53 cc. of ischemic kidney blood into each of six dogs led to an average blood pressure rise of 36 mm. Hg which was comparable to that following the injection of renin. Unlike renin, however, the ischemic kidney blood exerted its maximum pressor effect within one minute and subsided entirely in 13 minutes. The differences in the effect of these two substances cannot be explained upon a quantitative basis and it must be considered that the two substances are not completely identical.

The induction of renin tachyphylaxis in four nephrectomized dogs made the dogs unresponsive to the administration of ischemic kidney blood which prior to the induction of tachyphylaxis evoked a typical pressor response in these dogs. However, when two dogs were made insensitive to ischemic kidney blood by repeated injections of the latter, the injection of renin (1 mgm.) still provoked a pressor response which was comparable

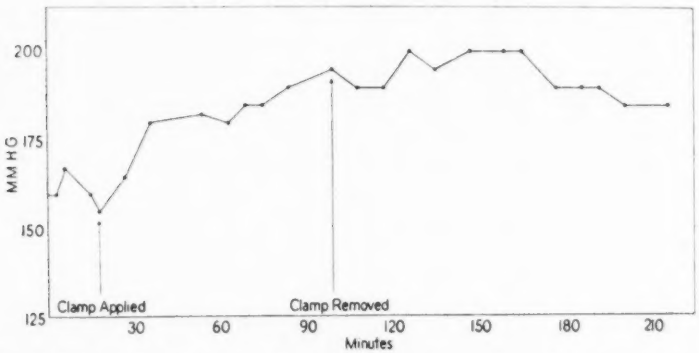

Fig. 2 (Expt. 1-J). The acute effect of unilateral partial renal ischemia of one remaining kidney on the blood pressure of a dog.

to that obtained before the induction of the ischemic kidney blood tachyphylaxis.

Properties of the pressor substance in ischemic kidney blood. 1. *The effect of heat.* In previous preliminary experiments it was found that the renal pressor substance was present in the plasma fraction of ischemic kidney blood. When four different samples of ischemic kidney blood containing the renal pressor substance were heated at 60°C. for 10 minutes, the typical pressor response was lost. For the heated ischemic kidney blood plasma produced a rapid, shortlasting rise (not over 2 min. in duration) in the blood pressure of the recipient dog, and this evanescent rise was followed by a prolonged decline in the blood pressure below the preinjection level. Normal plasma samples, when heated, produced this same type of pressor response when given to a recipient dog.

2. *The effect of dialysis upon ischemic kidney blood plasma.* Seven sam-

ples of heparinized plasma samples (25–50 cc.) obtained from ischemic kidney bloods, and five samples of normal plasma were dialyzed in cellophane bags against running water for 24 hours. At the end of this time, the contents of the bag were tested for pressor activity on the nephrectomized dog. It was found that there was a pressor response in the recipient dog following the administration of six of the seven dialyzed ischemic kidney blood plasma samples which averaged 32 mm. Hg, but there was no pressor response following the introduction of four of the five normal dialyzed plasma samples, and a rise of 10 mm. Hg. in the fifth sample. The pressor quality of the ischemic kidney blood plasma (dialyzed) was similar to that of the original ischemic kidney blood plasma both in the rapidity, intensity, and duration of the pressor effect.

3. *The effect of cocaine upon the the pressor action of ischemic kidney blood.* The prior intravenous administration of cocaine (20 mgm.) in four nephrectomized dogs did not prevent or diminish the pressor response following the administration of 50 cc. of ischemic kidney blood. The average pressor response following the introduction of the ischemic kidney blood (50 cc.) was 30 mm. Hg before and 38 mm. Hg after the administration of the cocaine in the four dogs observed.

III. THE LENGTH OF TIME NECESSARY FOR THE PRODUCTION OF THE RENAL PRESSOR SUBSTANCE FOLLOWING PARTIAL RENAL ISCHEMIA. In the second series of experiments partial renal ischemia was maintained for about four hours, but the time actually necessary for the production of the renal pressor substance was not known. Accordingly, the following experiments were performed.

Methods: The renal artery and vein of the normal anesthetized dog were isolated as described before. The Goldblatt clamp was applied, and the renal artery was severely constricted after 3 to 5 cc. of purified heparin had been given to the dog. The renal vein was then cannulated and the venous blood immediately collected in 15 minute samples at a maintained rate (by adjustment of the Goldblatt clamp) of 5 to 10 cc. per minute. At the end of an hour, the collections were discontinued. Four experiments were performed.

Fifty cubic centimeters of each 15 minute collection were then injected into the femoral vein of an anesthetized, previously nephrectomized dog (10–15 kgm.) and the pressor response was noted. It was found that the introduction of a similar quantity of either saline solution or normal dog's blood at the rate of injection used (25 cc/min.) did not cause a significant pressor response in the recipient dog.

Results: In the four experiments, it was observed that in each of the dogs renal venous blood collected during the first 15 minutes contained sufficient pressor material to evoke an average pressure rise of 35 mm. Hg when 50 cc. of it were given to another dog. Fifty cubic centimeter

quantities of the second, third and fourth 15 minute collections evoked average pressor responses of 35, 35 and 42 mm. Hg respectively in recipient dogs. Thus, the pressor potency of the first 15 minute collection of ischemic kidney blood was comparable not only to that of the later blood collections but also to that of venous blood collected after four hours of partial renal ischemia (see table 2).

IV. Neutralization of the renal pressor substance by the normal dog's kidney. Several observers (16, 17) have noted that the hypertension following the production of partial renal ischemia in the dog may be abolished or masked by the presence of a normally functioning kidney.

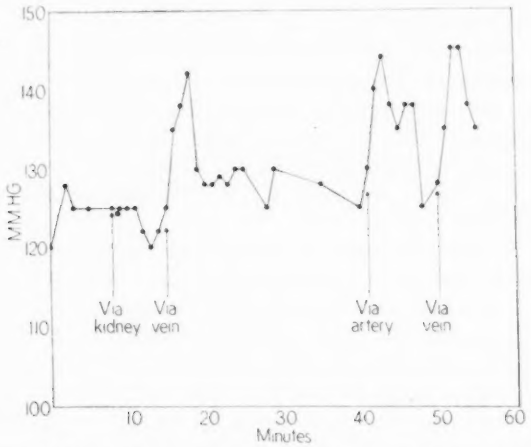

Fig. 3 (Expt. 13-A). The effect of the normal kidney on the pressor action of ischemic kidney blood. Fifty cubic centimeters of ischemic kidney blood given to recipient dog via—1, renal artery, 2, femoral vein, 3, femoral artery, and 4, femoral vein again.

Therefore it was thought advisable to determine the action of the intact and normally functioning kidney on the renal pressor substance.

Methods: The left renal artery of the normal, anesthetized dog was temporarily clamped in two places and cut between the clamps after 3 to 5 cc. of heparin had been given. Then, the cut ends of the artery were ligated around two barrels of a small three-barreled T-shaped cannula. The third barrel of the cannula was attached to a narrow, flexible rubber tube extending through the abdominal wall of the dog. This tube was temporarily clamped with a hemostat and then the clamps on the renal artery were released. The wound was closed and both the left femoral artery and vein were similarly cannulated. Thus, ischemic kidney blood could be injected by syringe into the left renal artery, the femoral artery

and the femoral vein of the same dog. Two minutes were taken for each injection. Seven such experiments were performed.

Results: In the seven experiments (see fig. 3), a marked difference was observed in the response of the same recipient dog to the same quantity of renal pressor substance when it was given via the renal artery and when it was given via the femoral artery or vein. For when 40 cc. of ischemic kidney blood were given via the renal artery, the average pressor response was 5 mm. Hg, (range 0–10 mm. Hg), whereas the introduction of an equal quantity of the same lot of ischemic kidney blood into the femoral artery and vein led to an average rise of 16 and 18 mm. Hg, respectively. It may be mentioned that in one experiment the introduction of the renal pressor substance via the splenic artery led to a well-marked pressure rise comparable in extent to that found when the substance was injected into the femoral vein.

SUMMARY AND CONCLUSIONS

In the preceding observations the presence of a pressor substance in the venous blood of either an isolated perfused kidney or of the intact kidney of the dog is reported. It was found that this substance could be detected in the blood leaving the partially ischemic kidney within 15 minutes after the initiation of the partial ischemia. Further, it was found that its pressor quality per cubic centimeter at this time was as strong as that found in blood obtained after a much longer period of partial ischemia This last observation indicates that the production of this substance is not necessarily dependent upon progressive autolysis or destruction of the kidney.

The ischemic kidney blood was found to have a pressor effect which differed from that of renin in that its action was immediate and of moderate duration. Further, animals made tachyphylactic to the introduction of ischemic kidney blood still reacted to the injection of renin. On the basis of these differences, it was felt that the pressor quality of the ischemic kidney blood was not due to the presence of renin alone.

The renal pressor substance was found to be nullified by the action of heat on the plasma containing it. It was also ineffective when injected into a dog via the renal artery supplying a normal kidney. However, the renal pressor substance could not be removed from ischemic blood plasma by prolonged dialysis of the latter nor was it ineffective when given to cocainized dogs.

Since this ischemic kidney blood contains a pressor substance that is not exactly similar to renin in its physiological actions and differs somewhat from "angiotonin" ("hypertensin") in some of its chemical properties, it is believed that the substance is not identical to either of them.

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